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INSTRUMENTAL ANALYSIS OF CHEMICAL POLLUTANTS

TRAINING MANUAL

ENVIRONMENTAL PROTECTION AGENCY

APRIL 1974

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This manual contains lecture and laboratory outlines dealing with the instrumental analysis of certain pollutants found in water; e.g., metals, chlorinated hydrocarbons and organophosphorus compounds.

Topics covered are sample collection and isolation, quality control, mass spectrometry, gas chromatography, infrared, emission spectroscopy and atomic absorption.

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INSTRUMENTAL ANALYSIS OF CHEMICAL POLLUTANTS

This course is designed for analytical chemists and others having technical responsibility for the collection, identification and measurement of organic and inorganic pollutants in the aquatic environment.

ENVIRONMENTAL PROTECTION AGENCY
Water Quality Office
TRAINING PROGRAM

APRIL 1974

IV

CONTENTS

Title or Description	Outline Number

I INTRODUCTION

Instrumentation in Water Chemistry	1
Automation of Chemical Analysis	2
The Problem of Identification in the Analysis of Organic Compounds	3
Sample Handling-Field Through Laboratory	4

II CHROMATOGRAPHIC METHODOLOGY

Carbon Adsorption Method Applied to Isolation of Pesticides From Water	5
Methods of Recovering Organic Materials From Surface Waters	6
Preliminary Separation of Extracts	7
Laboratory Procedures for the Separation of Extracts	8
Liquid-Liquid Extraction Methods for Recovery or Organic Substances from Streams	9
Thin Layer Chromatography	10
Introduction to Gas-Liquid Chromatography (Part I)	11
Basic Theory of Gas-Liquid Chromatography (Part II)	12
Theory of Gas Chromatography Peak Broadening	13
Characterisitics of Electron Capture and Other Ionization Detection Systems	14
Ionization Detectors	15
Thermal Conductivity	16
Special Detectors	17
Characterisitics of the Flame Ionization Detector	18
Preparing Gas Chromatography Columns	19
Trouble Shooting the Gas Chromatograph	20
Quantitation in Gas Chromatography	21

Title or Description | Outline Number

III OPTICAL METHODS (RADIATION) OF ANALYSIS

Recent Developments in the Identification of Oils by Instrumental Analysis (Part I) 22

Analyses of Oil Slicks (Part II) 23

Infrared Instrumentation 24

Infrared Identification of Organic Compounds 25

Analysis of Fish Tissue for Organochlorine Compounds 26

Atomic Absorption Spectrophotometry 27

Metals in Natural and Treated Waters in the Absence of Gross Pollution by Atomic Absorption Spectrophotometry 28

Principles of Emission Spectroscopy 29

Application of Emission Spectroscopy in Water Analysis 30

Applications of Integrated Multiparameter Water Quality Data Acquisition Systems in Pollution Surveillance 31

IV SPECIAL TOPICS

Extraction p-Values of Pesticides 32

Instrumental Analysis of Phenols and Mercaptans by Electron Capture Gas Chromatography 33

Special Instrumental Methods-Mass Spectrometry and NMR 34

Dosage and Process Control 35

Interlaboratory Quality Control Studies 36

Accuracy - Precision - Error 37

A Quick Look at Statistics 38

Aerial Reconnaissance in Pollution Surveillance 39

Case Preparation and Courtroom Procedure 40

V METHODOLOGY MANUAL

General Procedure for Pesticide Analysis 41

General Procedure for Atomic Absorption Analysis 42

Title or Description	Outline Number
Atomic Absorption Laboratory	43
Laboratory Procedure for the Determination of Lead by Atomic Absorption-Extraction Method	44
Analysis of Water Sample for Chlorinated Hydrocarbons	45
Analysis of Soil Sample for Chlorinated Hydrocarbon and Organophosphorus Compounds	46
Analysis of Soil Sample for Chlorinated Hydrocarbon and Organophosphorus Compounds	47
Analysis of Soil Sample for Chlorinated Hydrocarbon and Organophosphorus Compounds	48
Analysis of Soil Sample for Chlorinated Hydrocarbon and Organophosphorus Compounds	49
Analysis of Raw Water for Silvex (Rapid Screening Method)	50
Total Carbon Analysis	51
Carbon-Nitrogen-Hydrogen Analyzer	52

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INSTRUMENTATION IN WATER CHEMISTRY

I CHANGES IN WATER AND WASTE CHEMISTRY

A Water and Sewage Treatment Plants

1 In the past 40 years, treatment plants have increased in complexity and efficiency, utilizing new processes to accomplish a higher degree of treatment, in order to meet the need for expanded water use and to reduce water pollution.

2 Because of the sophisticated systems now in use, and the need for still further improvement in treatment, the associated analytical work to monitor the treatment processes and to evaluate the quality of the end product has become more difficult.

B Industrial Waste Analyses

1 Rapid industrial growth has greatly increased the problems of waste effluent characterization and the monitoring of the effects on receiving waters. Analytical procedures are now required for compounds which did not exist a few years ago.

2 The large increase in sources of industrial wastes has produced a significant increase in the workload of the average pollution control laboratory.

C Increased Analytical Requirements

1 The modern laboratory must utilize analytical procedures for a large number of specific pollutants, present in extremely small concentrations. For example, the 1936 edition of Standard Methods of Water Analysis lists approximately 40 determinations for water and wastes, while the 1965 edition includes more than 30 additional measurements which can be made. Toxicity studies are continually indicating the need to measure pollutants in the submicrogram range, because of subtle, long-term effects on aquatic and human populations.

2 To meet the current needs, laboratory instrumentation must be utilized wherever possible. In many cases, the required specificity, speed, and reliability cannot be accomplished by the older manual procedures. The use of automated equipment effectively reduces manpower requirements.

II ADVANTAGES AND LIMITATIONS OF INSTRUMENTS

A Advantages

1 Speed

Many instrumental methods are quite rapid when compared to traditional bench methods. For example, metals by atomic absorption can be determined at the rate of 30-50 per hour, while the corresponding colorimetric methods are very tedious and time-consuming. Autoanalyzer techniques permit 20-40 determinations per hour, as contrasted to 5-10 per hour by older methods. Separation techniques by gas chromatograph are extremely rapid.

2 Reduced error

When properly calibrated and maintained, instruments are much less subject to random manipulation error. The Autoanalyzer precision is markedly superior to even the best manual techniques, due to mechanical reporduction of sample measurement and reagent addition.

3 Data handling

Modern laboratory instruments often provide direct concentration readout, eliminating calculation. Automatic printing of final results is available for many instruments.

4 Automation

Instruments can often be adapted to incorporate automatic sampling, analysis, and final recording. When coupled to computers, the entire process through data storage can be automated.

B Limitations

1 Availability

Dependable instrumentation, at reasonable costs, is not available for many analyses performed in the water laboratory. For example, satisfactory instrument packages are not available for solids, biochemical oxygen demand, color, chemical oxygen demand, cyanides, phenolics, sulfide, or threshold odor.

2 Sample variation

Instruments cannot provide judgment. They cannot recognize or compensate for extreme changes in concentration, interfering substances, precipitates, or lack of proper sample preparation.

3 Lack of control

Most instruments are not equipped to indicate that the system is not working properly or that mechanical or electronic failures are producing erroneous results.

III DESIRABLE DESIGN CHARACTERISTICS

A Specificity and Adaptability

The instrument should actually measure the constituent or sample characteristic and nothing else. However, provision should be made to adapt the equipment to measure a number of similar elements or compounds.

B Rapidity

To utilize the advantages of speed, the instrument should be capable of performing complete analyses in a relatively short time, generally a few minutes.

C Ruggedness

The equipment should be able to maintain proper operation without frequent readjustment or recalibration and should withstand daily usage under normal laboratory conditions. Excess maintenance or frequent breakdown reduces the value of laboratory instruments.

D Data Processing

Ideally, the design should incorporate automatic calculation and final printout of the data in the appropriate concentration units.

IV QUALITY CONTROL OF INSTRUMENTAL ANALYSES

A Instrument Design

The most important factor in assuring reliable data in instrumental analyses is the design of the equipment. Within any class of laboratory instruments, design varies from very poor to excellent. In addition to basically similar circuitry or optics, some instruments provide better control of drift, simplified calibration or adjustment, superior readout, or useful accessories. The desirable features above should be considered in selecting instruments.

B Standardization

In the performance of instrumental measurements, calibration by means of known reference values is very important. The analyst should establish a routine calibration system and should determine by experimentation the required frequency of recalibration. In the case of strip-chart recording, digital presentation, or automatic printout, the entire system, including final data presentation, should be checked and calibrated.

C Controls

It is a serious mistake to assume that because an instrument was working properly it will continue to do so. The method used should include a sufficient number of standards, spiked samples, or other controls to establish satisfactory performance during routine operation. Since the instrument cannot distinguish a false value from a true one, the analyst must provide the needed control.

V NEEDS AND FUTURE DEVELOPMENTS

A Specific-Ion Electrodes

The recent development of specific-ion electrodes has provided a new analytical tool for the water and waste chemist. Research efforts to improve existing systems and to develop new electrodes should expand the use of this instrumental approach.

B Gas Chromatograph Detectors

New detector systems are needed to expand the usefulness of gas chromatography for organic pollutant measurements. Improved detectors for nitrogen, phosphorus, and hydrocarbons would increase the ability to detect and measure organic compounds containing these elements and structures.

C Automation

The current trend toward complete automation of laboratory tests will be beneficial to the analyst. While providing more rapid, repetitive determinations, increased automation will require greater attention to quality control procedures.

D Data Handling and Computer Interfacing

Many instruments are now being designed to provide digital concentration readout and machine printing. The next logical step will be direct connection to a small computer in the laboratory or suitable interfacing to a time-sharing facility. Such systems are now available for complex instruments such as mass spectrographs and NMR and will in the future be available for even the simplest laboratory instruments. For example, a computer system has been developed to continuously accept the signals from up to 30 gas chromatographs simultaneously and to provide printouts of the values for each instrument.

This outline was prepared D. G. Ballinger, Director, Analytical Quality Control Laboratory, Division of Research, WQO, 1014 Broadway, Cincinnati, OH 45202.

Descriptors: Chemical Analysis, Intrumentation, Pollutant Identification, Water Analysis, Water Measurement

AUTOMATION OF CHEMICAL ANALYSIS

I INTRODUCTION

Environmental pollution control and monitoring have become, in very recent years, the focal point of national and international attention. The analytical chemist has an important role in matters regarding pollution, for he determines not only the amount of pollution in air or water but also the efficiency of prevention and treatment methods. The increasing number of samples and of measurements to be made requires him to employ every means possible to keep abreast with the demands.

The need for instrumental and automated analytical techniques is well recognized. Automated sensors with continuous readout for the measurement of key constituents associated with pollution would be ideal.

II DEGREES OF AUTOMATION

A Maximum automation - continuous sampling direct from source, with telemetering of results to central computer.

B Medium automation - grab samples in laboratory with multiple simultaneous instrumental analysis, recording, calculating and digital print-out of data.

C Minimum automation - maximum simplicity and efficiency of manual analytical methods.

Possible considerations are:

1 Automatic pipettes, burettes and reagent dispensers

2 Choice of glassware - precision and accuracy of graduated test tubes and cylinders vs. volumetric flasks should be compared to the accuracy of the analytical method.

3 Electrical "on and off" timers on hot plates (C.O.D.) and automated instruments (Technicon).

4 Use of semi-micro methods:

- a TKN - digest and distill 50 ml sample in place of 500 ml.
- b B.O.D. - incubate 125 ml samples in place of 300 ml.

5 Combining reagents:

- a Nitrite - reagent contains buffer, diazotization and coupling reagent.
- b Phosphorus - reagent contains acid, molybdate and reductant.

6 Laboratory management - elimination of needless repetitious work

- a Lab's sample log number vs. engineer's coding system
- b Distributing samples to instrumental stations in permanent labeled containers
- c Permanent data sheets vs. individual slips of paper for record keeping.
- d Washing glassware - cleaning acid vs. soap; labor costs
- e Documented laboratory procedures, techniques, and practices to avoid variations.

III SENSORS AND INSTRUMENTATION

In order to measure the amount of any constituent in a liquid or gaseous medium on an automated system, a relationship between the parameter and an electrical signal must be established. When such a relationship is established and is not affected by uncontrollable variables, electronic technology already available can be applied to the sensor's signal for digital print-out of concentration or for telemetering and conversion of the signal to meaningful data through computerization.

Various types of sensors and instrumental methods for water quality parameters are shown in Table 1. Parameters whose measurement is based on an electrochemical principle are very desirable because of the possibility of developing an electrode or probe. In such a case, no intermediate chemical reaction is needed for the ion in the sample to produce a signal.

A Probes for many specific ions are available; however, most are affected by interferences and variables. The activity of the specific ion, sensed by the electrodes, is related to its concentration by the activity coefficient, which depends on the total ionic strength of the solution(4). By increasing the ionic strength in all the samples, the effect of individual variation between samples can be eliminated.

With some specific ion probes, a pH adjustor and a complexing agent must also be added to the samples to eliminate interference from hydrogen, hydroxyl and metal ions.

Specific ion probes are valuable in some applications but must be evaluated for such use. Most of the automated continuous monitoring systems for water quality that are now available use sensors which require no intermediate chemical reaction and only measure parameters such as temperature, pH, conductivity, solar radiation, turbidity, chlorides and dissolved oxygen.

B Automatic Titrators with preset end point are applicable for many measurements using various types of electrodes. Up to 10 samples can be titrated without attendance.

C Atomic Absorption - This method is applicable for determining the concentration of many metals. The instrument is usually used only in a laboratory. It is adaptable to:

1 an automatic sampler
2 automatic reagent additions
3 automatic extraction

D Photometric Methods - With automated addition of reagents, the end product can be measured with flow through cells in:

1 Visible range

2 Ultra violet range

E Organic Carbon Analyzer - Manual injection of samples vs. automated sampling system is dependent on amount of suspended solids in samples.

IV PRESERVATIVES AND QUALITY CONTROL

A Preservatives added to samples must be compatible with the automated method to be used. For example, sulfuric acid and mercury may interfere with colorimetric methods.

With automated methods, the preservative should be added to samples, standards and control blanks.

B Routine standardization of automated instrument - After every 10 or 20 samples:

1 Check baseline with a control blank

2 Check sensitivity with a standard

The laboratory should also establish a program for routine interlaboratory and intralaboratory quality control.

V AUTOMATED WET-CHEMICAL SYSTEMS

Many chemical parameters for water quality are defined by the conditions in the chemical analytical methodology of Standard Methods, e.g. C.O.D., chlorine, phenolics and TKN digestion. All new automated methods should be compared to the manual Standard Method for accuracy.

A Robot Chemist - individual sample and reaction vessel, syringe type measurement of reagents, micro-switches and values, complex mechanics. Many are not very well accepted by chemists.

B Mecolab - individual sample and reaction containers; maximum 15 samples without manual change

C Technicon AutoAnalyzer - a continuous flow through system propelled by depression of various inside diameters of tygon tubing using constant speed rollers. The modular system is capable of almost all manual chemical techniques, such as sampling (up to 200 samples), filtration, dilution, addition of reagents, mixing, heating, dialysis, steam distillation, extraction with an immiscible solvent and digestion. The concentration of the end products is measured with a photometer or fluorometer and recorded. The versatility of the AutoAnalyzer enables the automation of almost any manual procedure.

The success of each automated procedure is primarily dependent on the manifold design. Effort should be made to achieve the maximum rate of each chemical reaction by determining the optimum conditions of time, temperature, pH and reagent concentration. When this is obtained, there is generally an increase in sensitivity, thus requiring a smaller aliquot of sample with less chance of interfering ions, a shorter flow stream through the system which will produce a faster response and wash out (shorter sampling time) and smoother, steadier plateaus.

The Technicon AutoAnalyzer is now internationally used for automating wet-chemical procedures in the laboratory. The Analytical Quality Control Laboratory of EPA includes Technicon procedures in the manual(9) of methods for Office of Water Programs laboratories.

VI CONTINUOUS MONITORING SYSTEM

A Several criteria or objectives have been established for using the AutoAnalyzer for continuous monitoring of a stream or a treatment plant influent or effluent:

1 Capability of continuous operation for one week with a minimum amount of manual manipulation (less than 4 man hours per week).

2 At least a ± 5% accuracy at the 50% (0.30 absorbance) absorption concentration.

3 Reagents and base line should remain stable for a week.

4 Reagent consumption should be less than 4 liters per week.

5 There should be a minimum distance of reaction-flow stream for a fast response and equilibrium of the system.

6 It is desirable that colorimetric chemistry obeys Beer's Law for rapid calculations.

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(Continued on Page 35-5)

TABLE 1

INSTRUMENTAL METHODS FOR ANALYSIS OF WATER QUALITY PARAMETERS

PARAMETER	SENSOR	MODE OF MEASUREMENT	USES*	EVALUATION
TEMPERATURE	probe	thermacouple	P, F, L, C	excellent
pH	electrode	potentiometric	P, F, L, C	good
CONDUCTIVITY	electrode	conductivity	P, F, L, C	good
SOLAR RADIATION	photocell	photometric	P, F, L, C	excellent
DISSOLVED OXYGEN	electrode	polarographic	P, F, L, C	good
CHLORIDE	electrode	conductivity	P, F, L, C	fair
	wet-chemical	potentiometric photometric	F, L, C L	— —
TURBIDITY	photocell (scatter)	photometric	P, F, L, C	good
COLOR	photocell (absorbance)	photometric	F, L, C	good
SUSPENDED SOLIDS	photocell	photometric	—	—
ALKALINITY	wet-chemical	potentiometric	L	—
	wet-chemical	photometric	L	—
ACIDITY	wet-chemical	potentiometric	L	—
	wet-chemical	photometric	L	—
CALCIUM	electrode	potentiometric	P, L	—
	flame	atomic absorption	L	—
	wet-chemical	photometric	L	—
MAGNESIUM	flame	atomic absorption	L	—
	wet-chemical	photometric	L	—
AMMONIA	electrode	potentiometric	P, L, C(?)	—
	wet-chemical	photometric	P, L, C(?)	good
NITRITE	wet-chemical	photometric	P, L, C(?)	good
NITRATE	probe	potentiometric	—	—
	wet-chemical	photometric	P, L, C	good
PHOSPHORUS	wet-chemical	photometric	P, L, C	good
SULFATE	wet-chemical	photometric	L	—
SULFIDE	wet-chemical	photometric	L	—

TABLE 1 CONTINUED

INSTRUMENTAL METHODS FOR ANALYSIS OF WATER QUALITY PARAMETERS

PARAMETER	SENSOR	MODE OF MEASUREMENT	USES*	EVALUATION
TOTAL ORGANIC CARBON	wet-chemical	infrared	L	—
	wet-chemical	flame ionization	L	—
CYANIDE	electrode	potentiometric	P	—
	wet-chemical	photometric	P, L, C(?)	—
PHENOLICS	wet-chemical	photometric	—	—
SPECIFIC ORGANICS	gas-chromatography	electron-capture	L	—
	liquid-chromotography	photometric	L	—
BACTERIOLOGICAL	wet-enzyamatic	photometric	—	—
	wet-fluorescence	fluorometric	—	—

*F-Field, monitoring streams
P-Treatment Plants, monitoring influent or effluent
L-Laboratory, with individual or automated sampling system
C-Continuous operation, for monitoring streams or treatment plants for operational control.

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This outline was prepared by Lawrence J. Kamphake, Research Chemist, Waste Identification & Analysis Program, AWTRL, RATWRC, NERC, EPA, Cincinnati, OH 45268.

Descriptors: Analysis, Automation, Chemical Analysis, Instrumentation, Water Analysis

THE PROBLEM OF IDENTIFICATION IN THE ANALYSIS OF ORGANIC COMPOUNDS

I THE USES FOR ORGANIC ANALYSIS OF WATER CONTAMINANTS

A Quality Standards and Basic Data

1 Drinking Water: to discover causes of color, odor, taste. To detect health dangers in the form of toxicity, carcinogenesis, mutagenesis, teratogenesis.

2 Surface Waters: suitability for aquatic life and public water supply.

3 Wastes

a Industrial: to predict pollution effects; to meet requirements of discharge permit application and monitoring.

b Municipal: the same as for industrial, also to evaluate suitability for recycling for public and industrial uses.

B Operation Control, to Maintain Efficiency of Treatment of Wastes and Public Water Supplies

1 Control through monitoring removal of harmful organics.

2 Alertness against formation or inadvertent addition of new organics.

C Pollution Abatement

1 To develop Enforcement evidence to bring about injunctive or punitive action.

2 To monitor the polluter's timely compliance with terms of judgment or settlement.

II ORGANIC ANALYSIS AS A FORENSIC EXERCISE

A The Three Elements of a Forensic Problem (Kingston)

1 Reconstruction of Incidents: to find out who was involved and to collect adequate evidence to support the findings and conclusions. A reconstruction is an attempt to find the most probable sequence of actions that would lead to the observed evidence. Each observation should be explained by the reconstruction.

2 Identification of Materials: name the class or specific material. Example: identify the drug; identify human blood type (how far to go in sub-classification?). "Identification is the process of giving the material a name." "Many samples can be found all over the world which would be given the same name after an analysis of the same properties." "Identification arises from the need to prove the elements of a crime(violation)."

3 Individualization of Materials: the process of finding the source or origin of the material. A characteristic of criminalistics that also is inherent in pollution enforcement studies. Criminalistics depends much on physical evidence, bullet rifling, bolt marks on cartridges, fingerprints, etc. But also uses characteristics of composition, subtle blood types, paper chromatograms of ink, trace components of drugs, spectroscopy of paints, etc. "The more factors that can be shown to be identical, the greater their evidential value will be."

4 Consistency: to be convincing evidence, the reconstruction must account for all the evidence, both identification and individualization, and must not require evidence not found in the case.

B The Three Forensic Elements in Organic Chemical Research

1 Reconstruction is the theory tested or suspected reaction mechanism.

2 Identification of reaction products.

3 Individualization requires that the identified product can be proved to be produced from the theoretical starting chemicals (or theoretical intermediates). This proof is often done by use of isotope-labelled reactants.

C The Three Forensic Elements in a Pollution Enforcement Case

An added complexity: the "crime" is usually discovered at a distance from the place where it was committed. Examples: a fish kill far downstream from point of pesticide discharge; a phenol discharge is not detected till the water is processed for drinking water; in groundwater pollution we often do not even know the direction of pollutant travel. These problems make reconstruction the most important element; it comes after laboratory work discovers evidence.

1 Identification defines the presence of: a specific (or class of) pesticides; phenols (as a class based on analytical method); a petroleum product (crude oil, gasoline, kerosene, etc.).

2 Individualization not only narrows down the chemical characterization, but makes identification so detailed as to trace the pollutant to a specific source and prove they are identical.

3 Reconstruction has to prove that the accused source discharged the actual batch of pollutant recovered at the site of the effect (dead fish, oil on beaches, odor in water).

III SOME SUCCESSFUL EXAMPLES IN POLLUTION ENFORCEMENT

A Mississippi Fish Kill

1 Identification: endrin, by GC, IR, in tissue and water.

2 Individualization: compounds "X" and "Y" shown to be intermediates in manufacture; accompanying traces of heptachlor.

3 Reconstruction

a CCE's showed endrin occurence paralleled fish kills of earlier years.

b Compounds "X & Y" indicated the source to be a manufacturer, rather than a user.

c Heptachlor had to be accounted for.

d Only 2 manufacturers of endrin, only one with access to Mississippi.

e Discovery of tons of discharged endrin, compounds X & Y, in company's sewers.

f Discovery of heptachlor significance

B Santa Barbara Oil Seeps

1 Identification as on oil was obvious.

2 Individualization: group composition different from crude oils, even nearby. Problem: Could the difference be caused by weathering?

3 Reconstruction: Stimulated weathering did not give "seep oil" composition. Fresh sample by diver had same properties as floating oil. Fresh oil on beaches had same composition as oil floating above seep.

4 Special case: one floating sample shown to be refinery waste, mainly wax.

C Wright Field Jet Fuel Spill

1 Identification: as jet fuel (there are 5 or so different products all being essentially kerosene).

2 Individualization: characteristic pattern of low boilers; unusually low sulfur compounds.

3 Reconstruction: jet fuel and not other kerosenes. With enough reference material, this could have been shown to be military fuel, not civilian, could be matched against the actual tank allowed to spill.

D Other Selected Cases

1 Indianapolis Pyridines

2 New York State Police Well

3 New Bedford Tar Balls

4 Denver Earthquake

This outline was prepared by A. A. Rosen, Chief, Waste Identification and Analysis Activities, Advanced Waste Treatment Research Laboratory, WQO, Cincinnati, OH 45226

Descriptors: Organic Compounds, Organic Matter, Organic Wastes, Water Analysis

SAMPLE HANDLING - FIELD THROUGH LABORATORY

I PLANNING A SAMPLING PROGRAM

A Factors to Consider:

1 Locating sampling sites

2 Sampling equipment

3 Type of sample required

a grab
b composite

4 Amount of sample required

5 Frequency of collection

6 Preservation measures, if any

B Decisive Criteria

1 Nature of the sample source

2 Stability of constituent(s) to be measured

3 Ultimate use of data

II REPRESENTATIVE SAMPLES

If a sample is to provide meaningful and valid data about the parent population, it must be representative of the conditions existing in that parent source at the sampling location.

A The container should be rinsed two or three times with the water to be collected.

B Compositing Samples

1 For some sources, a composite of samples is made which will represent the average situation for stable constituents.

2 The nature of the constituent to be determined may require a series of separate samples.

C The equipment used to collect the sample is an important factor to consider. ASTM(1) has a detailed section on various sampling devices and techniques.

D Great care must be exercised when collecting samples in sludge or mud areas and near benthic deposits. No definite procedure can be given, but careful effort should be made to obtain a representative sample.

III SAMPLE IDENTIFICATION

A Each sample must be unmistakably identified, preferably with a tag or label. The required information should be planned in advance.

B An information form preprinted on the tags or labels provides uniformity of sample records, assists the sampler, and helps ensure that vital information will not be omitted.

C Useful Identification Information includes:

1 sample identity code
2 signature of sampler
3 signature of witness
4 description of sampling location detailed enough to accommodate reproducible sampling. (It may be more convenient to record the details in the field record book).
5 sampling equipment used
6 date of collection
7 time of collection
8 type of sample (grab or composite)
9 water temperature
10 sampling conditions such as weather, water level, flow rate of source, etc.
11 any preservative additions or techniques
12 record of any determinations done in the field
13 type of analyses to be done in laboratory

IV SAMPLE CONTAINERS

A Available Materials

1 glass
2 plastic
3 hard rubber

B Considerations

1 Nature of the sample - Organics attack polyethylene.

2 Nature of constituent(s) to be determined - Cations can adsorb readily on some plastics and on certain glassware. Metal or aluminum foil cap liners can interfere with metal analyses.

3 Preservatives to be used - Mineral acids attack some plastics.

4 Mailing Requirements - Containers should be large enough to allow extra volume for effects of temperature changes during transit. All caps should be securely in place. Glass containers must be protected against breakage. Styrofoam linings are useful for protecting glassware.

C Preliminary Check

Any question of possible interferences related to the sample container should be resolved before the study begins. A preliminary check should be made using corresponding sample materials, containers, preservatives and analysis.

D Cleaning

If new containers are to be used, preliminary cleaning is usually not necessary.

If the sample containers have been used previously, they should be carefully cleaned before use.

There are several cleaning methods available. Choosing the best method involves careful consideration of the nature of the sample and of the constituent(s) to be determined.

1 Phosphate detergents should not be used to clean containers for phosphorus samples.

2 Traces of dichromate cleaning solution will interfere with metal analyses.

E Storage

Sample containers should be stored and transported in a manner to assure their readiness for use.

V SAMPLE PRESERVATION

Every effort should be made to achieve the shortest possible interval between sample collection and analyses. If there must be a delay and it is long enough to produce significant changes in the sample, preservation measures are required.

At best, however, preservation efforts can only retard changes that inevitably continue after the sample is removed from the parent population.

A Functions

Methods of preservation are relatively limited. The primary functions of those employed are:

1 to retard biological action
2 to retard precipitation or the hydrolysis of chemical compounds and complexes
3 to reduce volatility of constituents

B General Methods

1 pH control - This affects precipitation of metals, salt formation and can inhibit bacterial action.

2 Chemical Addition - The choice of chemical depends on the change to be controlled.

Mercuric chloride is commonly used as a bacterial inhibitor. Disposal of the mercury-containing samples is a problem and efforts to find a substitute for this toxicant are underway.

To dispose of solutions of inorganic mercury salts, a recommended procedure is to capture and retain the mercury salts as the sulfide at a high pH. Several firms have tentatively agreed to accept the mercury sulfide for re-processing after preliminary conditions are met.(4)

3 Refrigeration and Freezing - This is the best preservation technique available, but it is not applicable to all types of samples. It is not always a practical technique for field operations.

C Specific Methods

The EPA Methods Manual(2) includes a table summarizing the holding times and preservation techniques for several analytical procedures. This information also can be found in the standard references(1, 2, 3) as part of the discussion of the determination of interest.

VI METHODS OF ANALYSIS

Standard reference books of analytical procedures to determine the physical and chemical characteristics of various types of water samples are available.

A EPA Methods Manual

The Analytical Quality Control Laboratory, Office of Research and Monitoring, Environmental Protection Agency, has published a manual of analytical procedures to be used in Office of Water Programs laboratories for the analysis of water and wastes. The title of this manual is "Methods for Chemical Analysis of Water and Wastes" (1971).

For some procedures, the analyst is referred to Standard Methods and/or to ASTM Standards.

B Standard Methods

The American Public Health Association, the American Water Works Association and the Water Pollution Control Federation prepare and publish a volume describing methods of water analysis. These include physical and chemical procedures. The title of this book is "Standard Methods for the Examination of Water and Wastewater", 13th edition, 1971.

C ASTM Standards

The American Society for Testing and Materials publishes an annual "book" of specifications and methods for testing materials. The "book" currently consists of 33 parts. The part applicable to water is a book titled, "Annual Book of ASTM Standards, Part 23, Water; Atmospheric Analysis".

D Other References

Current literature and other books of analytical procedures with related information are available to the analyst.

VII ORDER OF ANALYSES

The ideal situation is to perform all analyses shortly after sample collection. In the practical order, this is rarely possible. The allowable holding time for preserved samples is the basis for scheduling analyses.

A The allowable holding time for samples depends on the nature of the sample, the stability of the constituent(s) to be determined and the conditions of storage.

1 For some constituents and physical values, immediate determination is required, e.g. dissolved oxygen, pH.

2 Using preservation techniques, the holding times for other determinations range from 6 hours (BOD) to 7 days (COD). Metals may be held up to 6 months.(2).

3 The EPA Methods Manual(2) includes a table summarizing holding times and preservation techniques for several analytical procedures. This information can also be found in the standard

references[1,2,3] as part of the discussion of the determination of interest.

4 If dissolved concentrations are sought, filtration should be done in the field if at all possible. Otherwise, the sample is filtered as soon as it is received in the laboratory. A 0.45 micron membrane filter is recommended for reproducible filtration.

B The time interval between collection and analysis is important and should be recorded in the laboratory record book.

VIII RECORD KEEPING

The importance of maintaining a bound, legible record of pertinent information on samples cannot be over-emphasized.

A Field Operations

A bound notebook should be used. Sample information written in it would include:

1 Sample identification records (See Part III)

2 Any information requested by the analyst as significant

3 Details of sample preservation

4 A complete record of data on any determinations done in the field. (See B, next)

5 Shipping details and records

B Laboratory Operations

Samples should be logged in as soon as received and the analyses performed as soon as possible.

A bound notebook should be used. Preprinted data forms provide uniformity of records and help ensure that required information will be recorded. Such sheets should be permanently bound.

Items in the laboratory notebook would include:

1 sample identifying code
2 date and time of collection
3 date and time of analysis
4 the analytical method used
5 any deviations from the analytical method used
6 data obtained during analysis
7 quality control checks used
8 any information useful to those who interpret and use the data
9 signature of the analyst

IX SUMMARY

Valid data can be obtained only from a representative sample, unmistakably identified, carefully collected and stored. A skilled analyst, using reliable methods of analyses and performing the determinations within the prescribed time limits, can produce data for the sample. This data will be of value only if a written record exists to verify sample history from the field through the laboratory.

REFERENCES

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This outline was prepared by A. Donahue, Chemist, National Training Center, DTTB, MDS, OWP, EPA, Cincinnati, OH 45268.

Descriptors: Chemical Analysis, Marking Techniques, Sampling, Water Analysis

CARBON ADSORPTION METHOD APPLIED TO ISOLATION OF PESTICIDES FROM WATER

I INTRODUCTION

The purpose of this discussion is to present the concept of the carbon adsorption method (CAM) as it is applied to the isolation of pesticides, particularly the chlorinated hydrocarbon type, from water. Some of the principles, limitations, and utility of the method are presented. The method as described below is considered a qualitative technique. It was not designed specifically for the isolation of pesticides. Rather, it was designed as a general technique for isolation of a broad spectrum of organic chemicals from water. Coincidentally, we are able to isolate many important pesticides.

The CAM has been used as a routine surveillance tool by the Federal Water Pollution Control Administration's Water Quality Activities since 1957. Its value in this work has been proven. However, there are many factors to be considered before deciding to adopt the CAM. These factors will be discussed below.

The elaborate equipment described below may not be needed for specific problems. Small scale equipment may be applicable for specific problems. Bench tests should be carried out on small columns to evaluate the applicability of the method to the problem.

Details of the equipment used by FWPCA for collection and isolation of organics from water are not given here. This information is available in USDI Publication WP-22 (1966).

II COLLECTION OF SAMPLE

A Carbon Adsorption Cartridge

B Sampling Equipment

  1 High flow

  2 Low flow

  3 Finished water

C Total Volume and Rate of Flow

  1 Total volume

    5000 gallons
    1000 gallons
    300 gallons

  2 Rate of flow

    0.5 gpm
    0.25 gpm
    0.03 gpm

III PREPARATION OF THE SAMPLE

A Drying

B Extraction

C Separation and Clean-up

  1 Column chromatography

  2 Thin-layer chromatography

IV DETERMINATION

A Gas Chromatography

  1 Electron capture

  2 Microcoulometric titration

V SENSITIVITY LIMITS

All data derived by use of the CAM must be considered qualitative. Numerical values attached to results thus obtained are considered to be minimum values.

A Practical Limits

1 Gas chromatographic determination

Electron capture gas chromatography is capable of detecting absolute quantities of less than 1 nanogram (10^{-9}gm). The microcoulometric titration system (Model C-200, Dohrmann Instruments Company) provides a sensitivity of 5 to 10 nanograms for many pesticides.

Consider a 5000-gallon (20,000 liters) sample. The dilution factors involved in the analysis are 10 and 100. Thus:

1 ng × 10 × 100 = 1000 ng or 1 μg

The limit then is 1 μg/20,000 liters or 0.00005 μg/liter for the electron capture determination. The limit for the microcoulometric determination is estimated at 0.00025 to 0.0005 μg/l. In practical application the lower limit is conservatively estimated at 0.001 μg/l or 1 ppt for both systems.

B Theoretical Limits

Assuming freedom from interferences and with considerable additional effort, it is theoretically possible to detect concentrations of 0.00000005 to 0.00000025 μg/l in a 5000-gallon sample.

VI CONTROL OF INTERFERENCES

Control and awareness of interferences is extremely important in trace analysis. The great sensitivity of gas chromatographic instruments in pesticide analysis emphasizes this importance. It is essential, therefore, that the analyst take the ultimate precautions to minimize the problem.

There are several measures, preventive and remedial, that need to be taken in controlling interferences.

A Preventive

Examples of this type of measure are:

1 Storing carbon in sealed containers.

2 Prefiltering air which passes through carbon drying ovens.

3 Prohibiting the use of sprays for pest control in areas where the analyses are carried out.

B Remedial

1 Proper cleaning of all collection equipment prior to use.

2 Carbon blank determinations.

3 Solvent blank determinations and redistillation when required.

4 Silica gel blank determinations - column and TLC.

5 Proper cleaning and when possible, muffling of glassware.

All control determinations must be made under the conditions of the analysis, including the final step of determination on the gas chromatograph.

VII LIMITATIONS OF THE METHOD

There are certain limitations such as the cost of installation and maintenance of the elaborate collection equipment to be considered. The time required to collect a sample sometimes limits its usefulness. More important, however, are the limitations for quantitative recovery. Some of the factors which limit the method to a qualitative technique are listed below:

A Adsorption Efficiency

The adsorption efficiency may be affected by: the type of carbon, the rate of flow-contact time, the total volume of water sampled, preferential adsorption of some compounds, changes in selective adsorption during the collection of sample, overloading the carbon, pH, temperature, and mineral constituents in the water.

B Desorption Efficiency

The desorption efficiency may be affected by: the type of carbon, extracting solvent, extraction time, irreversible adsorption of some compounds.

C Chemical and Biological Decomposition During Adsorption and/or Desorption

D Prior Removal by Sand Pre-filter

The use of the new low flow, low throughput sampling units serves to minimize some of the limitations.

VIII ADVANTAGES OF THE METHOD

In spite of the foregoing limitations, the CAM has proved very useful in the continuous surveillance of organic contaminants in water. It provides two important advantages:

A A Continuous Integrated Sample Over a Period of Time

B A Large Volume Sample (Up to 5000 gal) Which Provides a Larger Quantity of Sample for Analyses

IX SUMMARY

The CAM as applied to pesticide analysis has been described. The need for control of interferences and the limitations of the method have been recognized. These variables suggest caution in the interpretation of data obtained by this technique. However, the method has important advantages over the small grab sample method which make it useful as a routine surveillance tool as well as in certain specific pollution problems.

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This outline was prepared by J. L. Lichtenberg, Chief, Organic Analyses Unit, Division of Research, Analytical Quality Control Laboratory, FWPCA, 1014 Broadway, Cincinnati, OH 45202.

Descriptors: Absorption, Activated Carbon, Adsorption, Carbon Chemical Analysis, Pesticide Residues, Pesticides, Water Analysis

METHODS OF RECOVERING ORGANIC MATERIALS FROM SURFACE WATERS

Methods of recovering organic materials from water may be classified according to the degree of concentration required before the desired analytical procedure can be applied.

I CONCENTRATED SOLUTIONS

When the concentration is high and/or the methods are sufficiently sensitive (i.e., when only a minor degree of concentration is involved), the following methods should be considered.

A Liquid-liquid extraction usually involves water and an immiscible organic solvent. Solvents should be investigated in the series of increasing polarity:

- Aliphatic hydrocarbons
- Aromatic hydrocarbons
- Ethers
- Chlorinated compounds
  - (CCl_4, $CHCl_3$, CH_2Cl_2)
- Esters
- Alcohols, amines, acids, etc.

Because of hydrolysis or decomposition, pH may be of critical importance; this point will be discussed at length in the discussion of Analytical Procedures. Inorganic salt concentration may also be important.

Remember the concept of the partition coefficient:

$$K = \frac{C_s}{C_w}$$

where K is the partition coefficient,

Cs is concentration in the extracting solvent

Cw is the concentration in water.

Continuous extractors must be used where the K value is not favorable to the extracting solvent. Continuous batch and counter-current extractors may be used.

Separatory funnels are available in a variety of sizes. Batch continuous extractors are commercially available in sizes up to one liter or so, and can be readily assembled in larger sizes. Continuous counter-current extractors are convenient for up to 10-20 gallons.

B Steam distillation can be used to strip a sample of small amounts of volatile organic compounds; in this case, the distillate usually must be further concentrated by liquid-liquid extraction. The organic compound should have at least a moderate vapor pressure at 100°C and should be almost insoluble in water.

A variation of this method is simple evaporation to concentrate non-volatile organic compounds.

C Precipitants may be used: silver salts of acids, chloroplatinates or tetraphenyl boron, derivatives of amines, phenylhydrazine derivatives of ketones and aldehydes, etc.

D Ion exchange may be used to concentrate acids and bases. The only limit to the volume of water to be filtered is the amount of inorganic salts in the water; these inorganic salts usually use up the exchange capacity too rapidly to make this method practicable.

II VERY DILUTE SOLUTIONS

To concentrate extremely dilute solutions, the carbon filter is the most useful method. The organic matter is adsorbed from aqueous solution and desorbed by an organic solvent. The great advantage is the large amount of water that can be put through a small filter; the disadvantages lie in the lack of quantitative adsorption and desorption.

A The Adsorption Process

1 The adsorption process involves an equilibrium in solution.

$$\text{Adsorbed} \rightleftarrows \text{Unadsorbed}$$

2 The Freundlich Isotherm is often useful in evaluating adsorption behavior.

$$\frac{x}{m} = kc^{\frac{1}{n}}$$

Units may be chosen for convenience, the larger the $\frac{1}{n}$, the stronger the adsorption. The constant k gives the $\frac{x}{m}$ value at unit concentration.

x = weight of adsorbed compound
m = weight of carbon
k = constant = x/m when c is unity
c = equilibrium concentration of organic compound in the liquid phase; it is measured directly.
$\frac{1}{n}$ = constant determined by the slope of the isotherm

3 General Rule: Non-polar compounds are strongly adsorbed and polar compounds weakly adsorbed from water (e.g., mineral oil is more strongly adsorbed than glycine).

4 Adsorption will depend on the type of carbon, grain size, pH of solution, polarity of adsorbate, nature of the solvent, temperature, etc. If powdered carbon is not used, the column length and contact time must be chosen so as to permit efficient adsorption.

5 Desorption is simply the reverse reaction in the adsorption equilibrium and is influenced by the same factors as adsorption. It is usually necessary to use an organic solvent for desorption with an extended period of extraction.

B Setting up the Carbon Filter

1 In the next section is a description of the procedure used in setting up and running carbon filters in our laboratory. However, it should be understood that elaborate apparatus is not required and that excellent results can be obtained with a piece of glass tubing closed at either end by a rubber stopper in which is inserted a piece of smaller glass tubing. Such apparatus is adequate for testing small volumes (e.g., sewage or concentrated industrial wastes), although not convenient for handling thousands of gallons of water.

2 Wherever the investigation is concerned only with certain specific compounds, bench tests in small columns should be carried out in order to evaluate the performance to be expected from the filter.

III INSTALLATION OF EQUIPMENT AND COLLECTION OF CARBON FILTER SAMPLES

A The carbon filter consists of a piece of pyrex glass pipe, 3" in diameter and 18" in length. The ends are fitted with brass plates and 3/4" galvanized nipples. A stainless steel screen is fixed in a neoprene gasket at both ends.

B Presettling, Prefilter, and Backwash

River water will frequently clog the carbon filter before the desired amount of water has been sampled. It is necessary to remove sufficient turbidity to permit the required amount of water to pass through the unit. This may require a presettling tank, and a prefilter containing sand and gravel. For waters having less than 100 ppm of turbidity, a presettling tank generally is not required. Tap water, of course, requires no prefiltering and may be passed directly into the carbon filter.

C Presettling Tank

A standard hot water tank connected with the inlet at the bottom and outlet at the top, with a clean-out tap at the bottom, can serve as a presettling tank. The outlet connects to the prefilter containing sand and gravel. The hot water tank should be flushed at frequent intervals to prevent large accumulations of solids. Open settling tanks can be used if care is taken to prevent long detention times and biological action. With open tanks a pump will be required to move the water through the filter.

D Sand Prefilter

The sand prefilter consists of a standard piece of steel pipe, 3" in diameter and 3 feet long, threaded at both ends and equipped with 3"×1" reducer couplings. Two disks of stainless steel screen are fitted to the inside diameter of the pipe. A simple way to prepare and hold the screen in place is to cut out a disk about the size of the outside diameter of the reducer and then push it into place so that is is below the threads. The screen is held tightly against the inside of the reducer. The pipe is packed with 6" of 1/8" gravel, 24 inches of 0.6 - 0.8 mm sand, and another 6" of 1/8" - 1/4" gravel. No free space is left in the pipe. The gravel should be packed by jarring while filling the pipe. This arrangement provides a strainer rather than a filter with a movable bed. With such an arrangement, backflushing can be done without disturbing the filter. The construction details are shown in Figure 1. The dimensions may be varied according to local conditions.

IV FILTER ARRANGEMENT

A The presettling tank, the sand prefilter, and the carbon filter should be installed at the most convenient source of raw water. If less than 15 psi pressure is available, it may be necessary to pump the water through the system. A schematic drawing of a workable system is shown in Figure 2. Exact lengths of pipes, etc., are not given since these will vary with the local situation. Both the sand and carbon filters should be connected with unions at both ends for easy removal. A typical installation is shown in Figure 2.

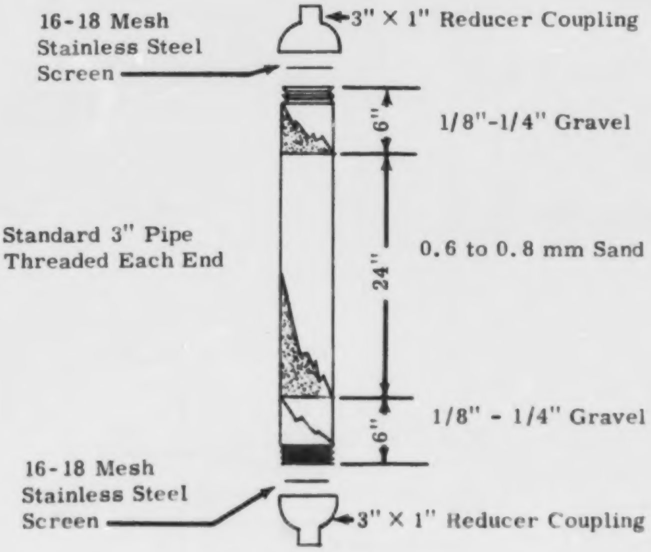


FIGURE 1 - Details of Sand Prefilter

B The raw water passes into the bottom of the sand prefilter, around into the bottom of the carbon filter. When the rate of flow through the system falls below 1/4 gpm, backwashing of the sand filter is necessary, using a high pressure source of water. A clean hose is connected to the top of the sand filter, the valve to the carbon filter is closed and the drain valve on the sand filter is opened. The sand is back-flushed until the water coming out is clear. The length of time between backwashings will vary. On the Missouri River, for example, it has been found that once every 24 hours is sufficient. After backwashing, connect the system as before and continue the sampling. Be sure to disconnect hose at top of sand filter after backwashing. A pressure gauge is inserted in the system to indicate when clogging is taking place in the carbon filter. Total pressure in the filter should not exceed 50 psi.

C A water meter located at the end of the system to prevent excessive fouling of the meter, is used to measure the volume of water samples. It is good practice to disassemble and clean the meter thoroughly after each run. A disk-type meter, 5/8"×3/4", is fairly satisfactory. A valve following the meter serves to throttle the flow, if necessary. A flow-regulating device may also be used for this purpose. The complete installation is shown in the schematic diagram in Figure 2.

D Fine carbon washes out of the filter when it is first started. A few gallons of water can be passed through the top connection and through the carbon filter drain before cutting in the meter to keep the meter free of carbon. The hose connection and drain on the carbon filter can be used to back-flush the carbon should it become clogged. This is a last resort and should only be used if absolutely necessary.

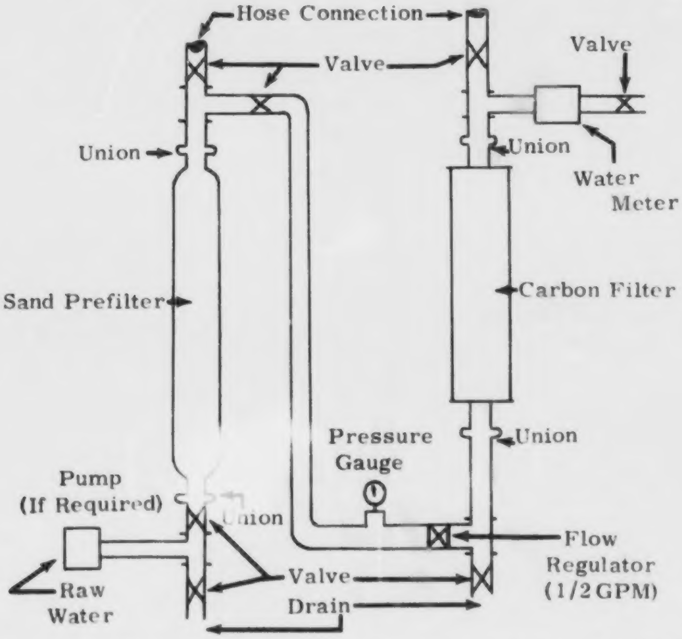


Figure 2 - Schematic Diagram of Piping Installation for Sand Prefilter and Carbon Filter

E The system outlined is not intended to remove all traces of turbidity from the water before passing through the carbon filter. Its purpose is to take out gross materials and most organisms, and to permit the required volume of water to pass through the carbon. The valve nearest the bottom of the sand filter is to be closed when it is desired to obtain a sample of raw water directly for other analyses. The drain valve is opened and, after flushing, the necessary raw water sample is collected.

F Pumping

1 If adequate pressure is not available for sampling raw water, it is necessary to pump through the filter system. It is important that the pump used should not contaminate the sample through oily packing or other sources. New pumps are oftentimes grease-coated and should be thoroughly cleaned before being put into service. If a lift of more than 12 feet is required, it will be necessary to use a jet-type pump. A model suitable for the conditions should be selected.

2 Before a new pump is put into service, it should be thoroughly flushed with hot water containing a little detergent. The pump should be operated against the minimum amount of head required to get the water through the filter. This may require by-passing of part of the flow.

G Collection of Sample

1 With fairly concentrated samples (sewage, industrial wastes, etc.), a small filter and a few gallons of water may be sufficient. Generally, for river sampling, water should be passed through a large filter at a rate of 1/4 - 1/2 gallon per minute, until up to 5000 gallons or more have been filtered. With highly turbid waters clogging may occur earlier. Although a suitable sample can be obtained sometimes with several hundred gallons of river water, it is desirable to filter a minimum of 2000 gallons if at all possible. A flow-regulating device set at 1/4 or 1/2 gpm can be placed in the system ahead of the carbon filter. A suitable unit can be obtained from Dole Valve Company, 6201 Oakton Street, Morton Grove, Illinois. These devices generally need a minimum pressure of 15 psi for proper operation.

2 Since the purpose is to get a quantitative measure of organics in water, it is very important to have accurate flow measurements. If difficulties in flow occur, this should be noted on a log sheet. Meter readings should be recorded daily on log sheets and should be designated either in GALLONS or CUBIC FEET. If a satisfactory meter can not be obtained, the flow rate for a set volume (1 or 2 liters) can be determined at regular intervals.

H Precautions

The purpose of the carbon filter is to adsorb small amounts of impurities from the water. It is important to avoid contamination of the carbon from other sources. Hence, the following should be observed.

1 New galvanized fittings are usually coated with oil or grease. The oil should be removed with a wash in kerosene followed by a detergent wash before such fittings are used for making connection to the filter.

2 Ordinary organic pipe jointing compounds should not be used. Red lead (lead oxide) mixed to a paste with water, can be used for this purpose.

3 Plastic hose is to be avoided. If rubber hose is used in any connections it should be flushed thoroughly before being connected to the filter. Copper tubing is ideal for connections.

I After the required volume of water has been run through the filter, the carbon is removed, dried, and extracted. The wet carbon is spread out in a thin layer on a metal sheet and air-dried for several days. The time needed for drying increases with the thickness of the layer. Warm air can be passed over the surface of the carbon to hasten the drying, but with the risk of driving off weakly held volatile substances.

V LABORATORY TREATMENT OF SAMPLES OBTAINED BY THE CARBON ADSORPTION TECHNIQUE: EXTRACTION PROCEDURES

The sample is collected by passing approximately 5000 gallons of water through a carbon adsorption unit. The unit is shipped to SEC, along with a daily record of the sampling activity and the carbon treated as described below.

A Preliminary Treatment

1 Records

- a Log sample in. List date received. Assign it a number.
- b Send daily record sheet to the laboratory where pertinent data is recorded on a data card (i.e., source, location, dates sampled, date received and total flow).

2 Drying

- a Remove wet carbon from the tube and dry it on copper, brass or stainless steel trays in an oven at 40°C. (n.b. Air circulated through drying cabinet should be prefiltered through carbon to prevent adsorption of foreign materials.) Drying requires about two days.
- b Store carbon samples in one gallon paint cans tightly closed.

B Solvent Extraction of Carbon

It is necessary that blanks be run on all solvents and on the carbon used for the collection of the sample.

1 Packing the extractors

- a Filter paper at bottom of soxhlet.
- b Glass wool (pre-extracted) 3" in depth to prevent carbon fines from passing into the pot. Add chloroform to wet the wool.
- c Carbon is added and packed. (n.b. Do not pack too tightly.)

2 Chloroform extraction

- a Add chloroform - about two cylinder volumes.
- b Extract for 35 hours.
- c Siphon and blow the bulk of the chloroform into the pot with air.
- d Remove the flask containing chloroform solution from the system. Concentrate to 250 ml by distillation and filter into a 300 ml Erlenmeyer flask. Evaporate to about 20 ml on a steam bath with a stream of air and transfer to a tared 5 dram vial. The remaining solvent is evaporated at room temperature without an air jet and the weight obtained.

3 Alcohol extraction

- a Remove residual chloroform from the carbon by one of the following methods.
  - 1) Blow warmed (40° C) prefiltered air upward through the carbon for about four hours.

2) Leach residual chloroform off the carbon with ethyl alcohol (95%) and distill off the chloroform alcohol mixture. Repeat until virtually pure alcohol remains.

3) Remove the carbon from the soxhlet and air dry on trays. When dry, repack into soxhlet as before. (Chloroform vapors must be disposed of.)

b Add sufficient ethanol (about two cylinder volumes) and extract for 24 hours.

c Concentrate the alcohol solution as in the case of chloroform, except that the final drying can be carried out on a steam bath with a stream of air.

The first method listed is the most satisfactory for our work here, mainly because a minimum of supervision is required and chloroform vapors are exhausted out the hoods.

C Extractable Materials

1 Calculation of concentrations

On most waters, it is convenient to compute the recovery in parts per billion (ppb) (i.e., $\mu g/l$), using the following formula.

$$\text{ppb} = \frac{\text{grams recovered} \times 10^6}{\text{gallons filtered} \times 3.785}$$

2 Infrared spectra

Infrared spectra are routinely run on both the chloroform and alcohol extracts.

D Special Applications

Other solvents may find use in performing extractions. If special applications are needed, a testing program is necessary to establish what solvents may best be used.

E Adsorption and Desorption

The effectiveness of adsorption and desorption varies for different materials and should be considered in interpretation of the results.

This outline was prepared by R.H. Burtschell, Chemist, formerly with AWTL, NERC, Cincinnati, OH and J. J. Lichtenberg, Pesticides Identification Group Leader, AQCL, NERC, EPA, Cincinnati, OH 45268

Descriptors: Absorption, Adsorption, Carbon Filters, Filtration, Organic Matter, Separation Techniques, Solvent Extractions, Water Analysis

PRELIMINARY SEPARATION OF EXTRACTS

I INTRODUCTION

As a general principle, it may be stated that the more sensitive modern analytical techniques (gas and other forms of chromatography, spectrophotometry, etc.,) require fairly pure samples to begin with. Also as a general principle, it may be said that the more widely different the purification steps employed, the greater the degree of purification.

Ion exchange, dialysis, crystallization, electrophoresis, etc., are useful but cannot be discussed here. We will cover briefly only three techniques.

A Distillation

B Partition

C Adsorption

II THEORETICAL BACKGROUND

A Distillation

1 Raoult's Law states that the partial pressure of A(P_A) in an ideal mixture of volatile solvents is

P_A = mole fraction of A in liquid × vapor pressure of pure A at the temperature of the system.

The total vapor pressure (which, of course, sets the boiling point) is then $P_A + P_B + \ldots$. This is the basis for studies of distillation. The question of non-ideal solutions is too complicated to discuss. Most physical chemistry texts go into it at length.

2 Partially miscible liquids are a special case, often discussed as "steam distillation" although co-distillation is a better term. Quoting from Glasstone, "As long as the two layers are present, irrespective of their relative amounts, the total vapor pressure remains constant and the system boils at a definite temperature."

Besides the familiar case of steam distillation, non-polar organics can be co-distilled with polar liquids, e.g., insecticides with glycerol and polar compounds with non-polar solvents (i.e., mineral oil). The co-distillation can be done with superheated solvents, under vacuum. Where trace quantities of one component are involved, there may not be a two-phase system present and the solvent vapor serves only as a carrier gas. Here the solute can usually be recovered easily, however, so the method is worth consideration regardless of the mechanism.

In practical work, the three important points are the stability of the sample, its volatility, and the ease of recovery from the distillate.

3 Distillation from acid and basic solutions may also be useful. The principle behind this will be discussed in the solubility separation method.

B Partition

Partition is the principle utilized in liquid-liquid extraction, paper chromatography and gas chromatography. The fundamental law is the Distribution Law: "A dissolved substance distributes itself between the layers of a two-layer system so that at constant temperature the ratio of the concentrations is also constant". The Law is properly applied only to dilute solutions, but a first approximation to the distribution ratio can be obtained from the ratio of solubilities.

$$K = \frac{C_s}{C_w} \qquad K = \frac{S_s}{S_w}$$

K is a true constant but apparent discrepancies occur where the solute has different molecular weights in the two phases. Benzoic acid, for instance, is largely ϕCOOH plus some benzoate ion in water; in benzene it is the dimer $(\phi COOH)_2$. K then applies only to the species common to both layers, ϕCOOH, and ignores the dimer.

Most texts on qualitative organic analysis contain a great deal of practical information on solubilities, while the usual undergraduate physical chemistry texts cover the essentials of the theory.

C Adsorption

There are two kinds of adsorption to be considered: physical (van der Waals) adsorption, and chemisorption. The theory of adsorption from solution is not as well understood as adsorption of gases but we can make a few comments.

Physical adsorption depends on the relatively weak van der Waals forces due to electrostatic attraction of dipoles, it being assumed that non-polar molecules act as oscillating dipoles; i.e., the nuclei of atoms and molecules form oscillating dipoles with the electrons. Since the bonds are weak, adsorption can be made reversible under proper conditions.

Chemisorption is thought of as actual chemical reaction, and the resulting bonds are much more difficult to break. Losses due to "irreversible adsorption" may therefore appear; this is particularly true of carbon, less so of silica and alumina.

III SOLUBILITY SEPARATION OF EXTRACTS

In the next section, we will describe a laboratory procedure which we have found very useful in handling carbon filter extracts. The extracts are split into acid, basic, and neutral fractions, and the neutral fraction is further separated by adsorption chromatography.

This procedure has been extremely useful where the fractions are weighed and infrared spectra made. However, where volatile compounds are to be looked for by gas chromatography, it is almost imperative that a steam distillation step be included. Otherwise the mass of heavy, probably polymeric, material present causes much interference.

Really good clean-up methods are likely to be highly specific and need to be "tailor-made" to suit the situation. Since this lecture is concerned only with the "preliminary" purification, we have omitted a discussion of precise analytical partition and adsorption columns (thin-layer, ion exchange, paper and gas chromatography, gradient elution methods, etc.) Such methods constitute a second and higher degree of purification, but ordinarily require that they be preceded by a "rougher" primary purification.

IV NOTE ON LOSSES OF VOLATILE COMPOUNDS

It may not be generally recognized, but it is quite true that heavy losses of even moderately volatile compounds occur if one tries to evaporate off all of the solvent when preparing to weigh an extract. We have had losses of over 50% in attempting to carry out what we thought were very careful evaporations of ether from 10 to 20 mg amounts of phenols.

For this reason, we suggest that no extract or fraction thereof be evaporated down for weighing unless it is known to be completely non-volatile. This precaution becomes more necessary as the purity of the sample increases, because non-volatile impurities act as "solvents" to reduce the amount of loss (according to Raoult's Law).

REFERENCES

1 Maron and Prutton. Principles of Physical Chemistry (4th Edition) · The Macmillan Co. 1965.

2 Moore. Physical Chemistry (3rd Ed.) Prentice Hall, Inc. 1965.

3 Glasstone and Lewis. Elements of Physical Chemistry. D. van Nostrand Co. 1960.

This outline was prepared by R. H Burtschell, Chemist, formerly with Waste Identification and Analysis Program, Advanced Waste Treatment Research Laboratory, NERC, EPA, Cincinnati, OH 45268.

Descriptors: Absortpion, Adsorption, Distillation, Organic Matter, Separation Techniques, Water Analysis

LABORATORY PROCEDURES FOR THE PRELIMINARY SEPARATION OF EXTRACTS

I STEAM DISTILLATION OF EXTRACTS

Steam distillation need be only a very simple procedure. Put a gram or two of crude or partially purified sample in a small flask containing 50 - 100 ml of distilled water. Set up for ordinary distillation and boil off most of the water. Set up for ordinary distillation and boil off most of the water. If necessary, add more water to the flask and continue distilling until the distillate has no tract of cloudiness or a second phase as it drips from the condenser. Transfer the distillate to a separatory funnel, extract with a suitable solvent, and concentrate to a convenient volume. As the product is relatively volatile, do not try to evaporate to dryness if it can be avoided. The non-volatile residue in the distillation flask can also be recovered if necessary.

If it is desired to recover acids (e.g., phenols in the 4-AAP colorimetric method), make the solution acid before distilling; the distillate will then contain only neutral and acidic substances. The neutrals can then be separated during the extraction step. Basic materials remaining in the still pot, e.g., aniline, can then be recovered by making basic and distilling over a second fraction.

II SOLUBILITY SEPARATION OF EXTRACTS

A The organic contaminants from water as extracted from carbon or concentrated by liquid extraction ordinarily form an exceedingly complex mixture for which there is no one satisfactory separation procedure. If the analysis is directed towards a single component, the procedure may be designed for that purpose; otherwise a useful and generally applicable preliminary separation may be made on the basis of relative acidities.

B By extracting an ether solution of the sample with water, then with dilute hydrochloric acid, then sodium bicarbonate, and finally sodium hydroxide, a separation into water soluble, basic, strongly acidic, weakly acidic, and neutral fractions may be made. The portion insoluble in ether may also be recovered as an additional fraction. This method is obviously not suited to very volatile substances, nor to further separation of the water soluble fraction, nor to substances unstable in the presence of water or dilute acid or base; in addition, it must be remembered that substances whose partition coefficients are not extremely unfavorable to water may be present in several fractions in varying amounts.

C This procedure constitutes one of the best preliminary steps in analyzing any unknown sample and may often profitably be either preceded or followed by steam distillation (at various pH's) fractional crystallization, etc. Simple micro qualitative tests for nitrogen, halogens, sulfur, phosphorus, etc., are also often useful; for a more extended discussion, see the texts mentioned in the bibliography.

D General

The sample should be substantially free of solvent. The amount may vary considerably but one-half gram is a very convenient amount; as little as 100 mg or even less may be used, but in this case there may be large percentage errors. Ethyl ether has proven to be an excellent solvent although often not all the sample will dissolve in it; benzene or chloroform may be used although these solvents are more likely to cause troublesome emulsions.

III LABORATORY DIRECTIONS

A Solubility Separation Procedure

Solution in Ether

1 Weigh previously dried sample contained in a tared 50 ml beaker (approximately 0.5 gm preferred). Also, weigh a 125 ml flask for the Water Solubles (WS).

2 Dissolve the sample with a small amount of methanol (about 1 ml). Add the methanol dropwise and stir with a rigid wire until the sample reaches a syrupy consistency.

3 Add 30 ml ether to the sample. Not all will dissolve. Collect the Ether Insolubles (EI) on a sintered glass funnel with suction and set aside for paragraph 4. Pour the contents of the suction flask into a 125 ml separatory funnel equipped with a teflon stopcock. (Stopcock grease will contaminate the sample.)

Ether Insolubles (EI)

4 Dissolve "EI" previously collected on the sintered glass funnel with methanol. Wash funnel once with a small volume of chloroform and collect in the suction flask. Transfer filtrate to the original tared beaker with methanol. Evaporate to dryness on a steam bath and record weight for Ether Insolubles.

Separation of the Water Solubles (WS)

5 Shake the ether solution(3) three times with 15 ml portions of distilled water. Drain the water layers into the weighed "WS" flask after each shaking. Evaporate the Water Soluble fraction to dryness on a steam bath with a jet of clean, dry air and record weight for Water Solubles.

Separation of Amine Fraction (B)

6 Mark flask "HCl". Shake ether solution three times with 15 ml portions of 5% HCl and drain aqueous layers into HCl flask after each shaking. Make this aqueous solution strongly basic by carefully adding NaOH pellets (about 30) or 25% NaOH. The solution becomes darker at this point. Set aside for back-extraction.

Separation of Strong Acid Fraction (SA)

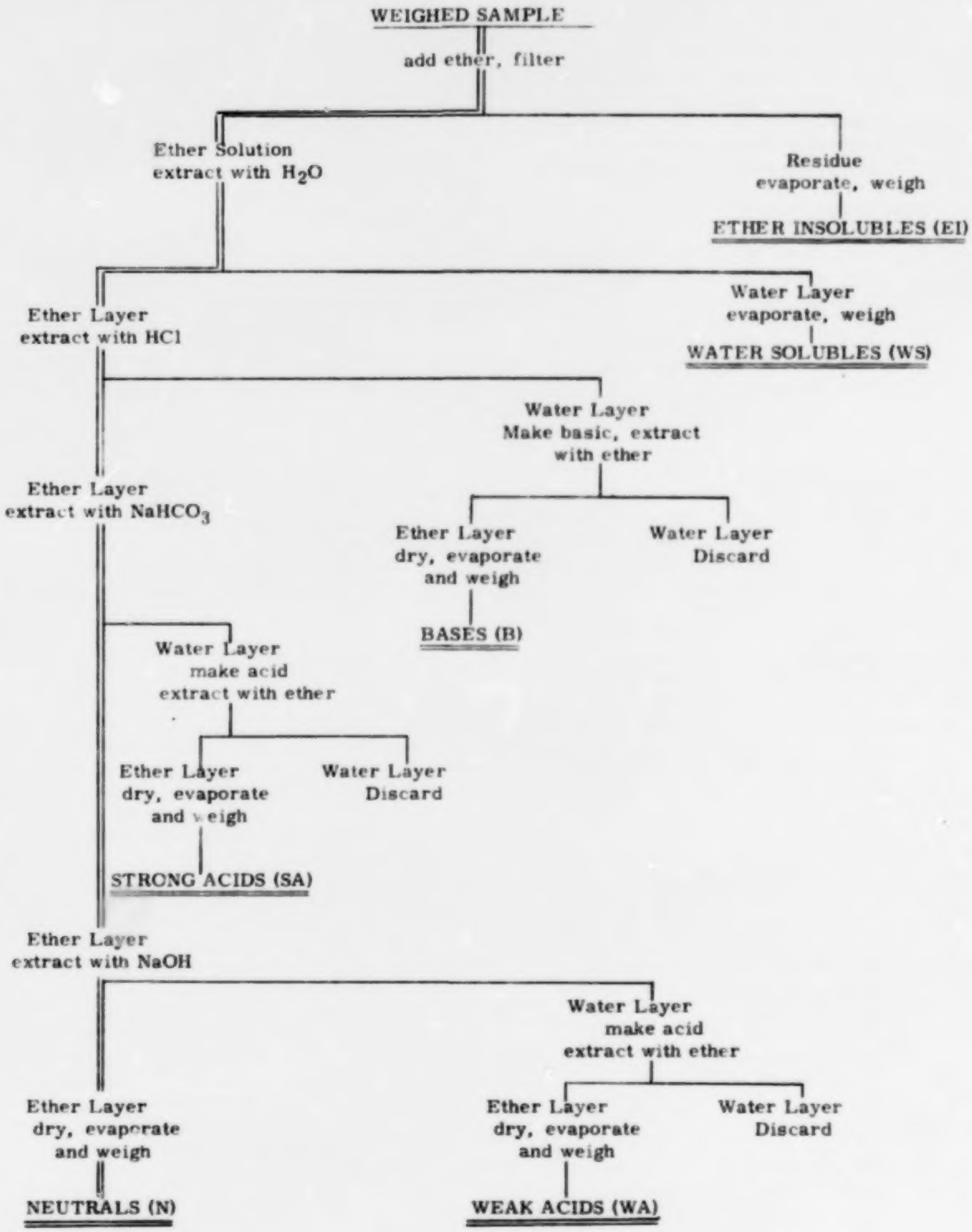
7 Restore, original ether volume to 30 ml, if necessary. Mark flask "$NaHCO_3$". Shake three times with 15 ml portions of 5% $NaHCO_3$ and drain aqueous layers into $NaHCO_3$ flask after each shaking. Acidify aqueous solution in flask by adding sufficient concentrated HCl (dropwise because of liberation of CO_2) until strongly acid to litmus paper. About 4 ml is sufficient. The solution becomes cloudy at this point. Set aside for back-extraction.

Separation of Weak Acid Fraction (WA)

8 Restore original ether volume to 30 ml, if necessary. Mark flask "NaOH". Shake three times with 15 ml portions of 5% NaOH and drain aqueous layers into "NaOH" flask after each shaking. Wash once with 15 ml distilled water and drain this into "NaOH" flask.

CAUTION: Tilt separatory funnel gently a few times, instead of shaking the first portion, as emulsions often occur.

If a small amount of the water wash remains emulsified, after draining, add several drops of saturated Na_2SO_4 solution and shake. This may help to break


WEIGHED SAMPLE
add ether, filter
Ether Solution
extract with H_2O
Residue
evaporate, weigh
ETHER INSOLUBLES (EI)
Ether Layer
extract with HCl
Water Layer
evaporate, weigh
WATER SOLUBLES (WS)
Water Layer
Make basic, extract with ether
Ether Layer
extract with $NaHCO_3$
Ether Layer
dry, evaporate and weigh
Water Layer
Discard
BASES (B)
Water Layer
make acid
extract with ether
Ether Layer
dry, evaporate and weigh
Water Layer
Discard
STRONG ACIDS (SA)
Ether Layer
extract with NaOH
Water Layer
make acid
extract with ether
Ether Layer
dry, evaporate and weigh
Ether Layer
dry, evaporate and weigh
Water Layer
Discard
NEUTRALS (N)
WEAK ACIDS (WA)

the emulsion. Acidify aqueous solution in flask with concentrated HCl until strongly acid to litmus paper. About 8 ml sufficient. The solution becomes cloudy at this point. Set aside for back-extraction.

Neutral Fraction (N)

9 Mark flask "N". Pour the ether layer remaining in the separatory funnel into the flask "N". Add about 10 gm of anhydrous sodium sulfate, cap with aluminum foil and allow to stand overnight to dry the ether solution.

B Back Extraction

Strong Acids (SA)

10 Mark flask "SA". Transfer $NaHCO_3$ extract from step 7 to separatory funnel and shake several times, carefully, relieving pressure caused by evolution of CO_2. Shake the $NaHCO_3$ extract, previously made acid, with a 15 ml portion of ether. Caution: Release CO_2 during first shaking repeatedly to avoid pressure build-up.

Drain the aqueous layer into the $NaHCO_3$ flask. Pour the ether layer into the "SA" flask. Return aqueous layer to separatory funnel and repeat extraction two more times with 15 ml portions of ether.

After all the ether layers have been collected in the "SA" flask, add about 10 gms anhydrous sodium sulfate, cap with foil and allow to stand overnight to dry the ether solution. Discard the aqueous $NaHCO_3$ portion which should be almost colorless.

Weak Acids (WA)

11 Mark flask "WA". Shake the NaOH extract, previously made acid, three times with 15 ml portions of ether and collect in flask "WA" as in step 10. Add sodium sulfate to dry, cap with foil and allow to stand. Discard aqueous NaOH portion which should be almost colorless.

Bases (B)

12 Mark flask "B". Shake the HCl extract, previously made basic, three times with 15 ml portions of ether and collect in flask "B". Add sodium sulfate to dry, cap with foil and allow to stand. Discard aqueous HCl portion.

C Transfer

13 Weigh four flasks and mark them "N", "WA", "SA", and "B". Record weights of each. Transfer the corresponding dried ether solutions from the Na_2SO_4 into the weighed flasks by filtering through filter paper.

14 Evaporate the ether from the above fractions with the aid of clean, dry circulating air and gentle heat on a steam bath. The ether solutions should be removed from the air and heat before totally dry and allowed to dry spontaneously to prevent loss of volatile components. Record the weight of the dried fractions.

D Chromatographic Separation

15 Weigh a 10 ml beaker and record weight. Dissolve the Neutrals in about 5 ml anhydrous ether, transfer most of the Neutrals to the beaker and place the remaining few drops in a vial for infrared analysis. Wash the (N) flask with ether and pour washings into vial also. Dry the Neutrals contained in the beaker by spontaneous evaporation and record weight.

16 Weigh a set of 125 ml Erlenmeyer flasks and mark them Aliphatics, Aromatics and Oxys. Record weights of each.

17 Fill the chromatographic column with 4 1/2" of silica gel and tap down to about 4". Place the Aliphatics flask under the column. Add about 20 ml of iso-octane to wet the column.

Aliphatics

18 Before the iso-octane reaches the level of the silica gel, add the Neutrals which have been previously adsorbed onto a small amount of silica gel by stirring in the beaker with a rigid wire. Elute with 85 ml iso-octane. Rinse the beaker with a small portion and add to the column first. Allow this volume to enter silica gel before adding the remainder of the iso-octane. Collect the eluent in the Aliphatics flask, evaporate to dryness with the aid of gentle heat and circulating air.

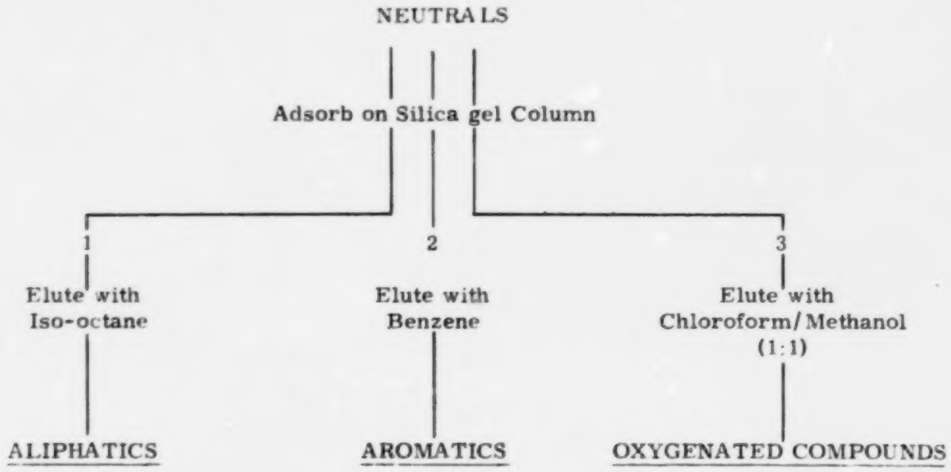
Aromatics

19 Place the Aromatics flask under the column. Elute column with 85 ml of Benzene-rinsing the beaker with a small portion and adding immediately as the previous eluent reaches the level of the silica gel. Allow this volume to enter silica gel before adding remainder of benzene. Evaporate with the aid of gentle heat and circulating air. The Aromatics fraction should be removed from the air and heat before totally dry and allowed to dry spontaneously to prevent loss of volatile components.

Oxys

20 Place the Oxys flask under the column. Elute with 85 ml of a 1:1 methanol chloroform mixture - rinsing the beaker with a small portion and proceed as before. Evaporate to dryness with the aid of gentle heat and circulating air.

CHROMATOGRAPHIC SEPARATION OF NEUTRALS



21 Record weights of the dried Aliphatics, Aromatics and Oxys.

NOTE: (All chromatographic solvents should be redistilled before use)

REFERENCES

1 Shriner, Fuson and Curtin. The Systematic Identification of Organic Compounds. 4th ed. John Wiley & Sons. New York. 1956.

2 Cheronis and Entrikin. Semimicro Qualitative Organic Analysis. Thomas Y. Crowell Co. New York. 1947.

3 Cheronis. Micro and Semimicro Methods. Vol. VI of the series Techniques of Organic Chemistry. Arnold Weissberger, Ed. Interscience. New York. 1954.

4 Schneider. Qualitative Organic Microanalysis. John Wiley & Sons. New York. 1946.

These books deal principally with chemical methods of identification; Shriner, Fuson and Curtin and Cheronis and Entrikin are recommended for those new to the field. For instrumental analysis and quantitative work, see standard works in those fields.

This outline was prepared by R. H. Burtschell, Chemist, formerly with AWTL, NERC, Cincinnati, OH and J. J. Lichtenberg, Pesticides Identification Group Leader, AQCL, NERC, EPA, Cincinnati, OH 45268.

Descriptors: Laboratory Tests, Organic Matter, Separation Techniques, Solvent Extractions, Water Analysis

LIQUID - LIQUID EXTRACTION METHODS FOR RECOVERY OF ORGANIC SUBSTANCES FROM STREAMS

I GENERAL PRINCIPLES

Liquid extraction is familiar to all chemists in the form of separatory funnel operations. A water solution of some organic substance is shaken with ether, the two liquid layers are allowed to separate, the water layer is discarded and the desired organic is recovered in the ether layer. A transfer of material has been accomplished from one liquid phase to another. This transfer is not instantaneous. The two liquids are brought into close contact by shaking actively for several minutes, until equilibrium is reached.

The condition at equilibrium is determined by a property of the solute, the partition coefficient:

$$K = \frac{C_s}{C_w}$$

Where: K is the partition coefficient

C_s is the concentration in the extracting solvent

C_w is the concentration in water

In the ideal situation, the value of K is fixed whether both liquids are saturated or are extremely dilute (for any given pair of solvents at a fixed temperature).

The portion of the solute transferred depends also on the relative volumes of the two liquids.

$$\frac{\text{Solute in organic layer}}{\text{Solute in water}} = \frac{C_s}{C_w}\,\frac{V_s}{V_w} = K\,\frac{V_s}{V_w}$$

Where: V_s is the volume of extracting solvent

V_w is the volume of water

Obviously, $C_sV_s + C_wV_w$ is the total weight of solute.

II BATCH EXTRACTION - SINGLE STAGE

Consider an ester with a partition coefficient: K = 1000 between hexane and water.

Now let us extract 20 liters of water containing 1 ppm ester with 100 ml of hexane.

$$\frac{\text{Solute in hexane}}{\text{Solute in } H_2O} = K\,\frac{V_s}{V_w} = 1000 \times \frac{100}{20,000} = \frac{5}{1} \text{ for an 83\% recovery.}$$

Batch extraction can be extremely useful in cases where the partition coefficient is very favorable. The following example deals with parathion with a K value assumed to be approximately 2000 between hexane and water.

If we extract one liter of water saturated with the insecticide, using 100 ml of hexane, then:

$$\frac{\text{Parathion in hexane}}{\text{Parathion in water}} = 2,000 \times \frac{100}{1000} = \frac{200}{1}$$

which is essentially quantitative.

Many chlorinated insecticides have partition coefficients even more favorable than in the example above. Since there are specific analytical methods of high sensitivity for many of these insecticides, it is practical to use batch extraction to prepare the sample for analysis. Depending on the analytical procedure and the concentration, water samples ranging from a liter to five gallons have been employed. Securing equilibrium is the main problem.

The equipment required for batch liquid extraction is quite simple. In many cases, separatory funnels ranging in capacity up to five liters may be used. Modifications may be devised for larger samples. A convenient device is a five-gallon carboy, using a solvent heavier than water, such as chloroform. Contact between the two liquids is accomplished by vigorous mechanical stirring, then the two phases are allowed to separate and the water is removed by decantation. The chloroform is transferred to a separatory funnel, where the removal of water is completed.

Increased extraction efficiency can be attained by performing multiple extractions of the sample with successive lots of solvent.

III BATCH EXTRACTION - CONTINUOUS

When the partition coefficient is not very favorable, extraction efficiency can be increased by using larger volumes of extraction solvent. One procedure was described in the preceding section. The continuous extractor is a device to accomplish the same result while limiting the solvent to a convenient volume.

In the continuous extractor, a relatively small volume of solvent circulates first through the water sample, where the extraction occurs, then to a distilling flask where pure solvent is distilled and passes back to the extractor. The extract concentrates in the distilling flask. Thus, it is possible to carry out the extraction with what in effect is a large volume of solvent and yet concentrate the extract in only a little solvent. It is evident that for this method the extract must be much less volatile than the solvent, or it will circulate, too.

Batch continuous extraction is rarely useful in water pollution studies, because the amount of water sample that it can be applied to is usually much too small. But the equipment can be operated for long periods with little attention and thus has been used to perform exhaustive extractions on a small scale for specialized analyses, e.g., with sewage.

A large device for several stages of extraction and up to 135 liters has been described by Kahn & Wayman; see Anal. Chem. 36, 1340. (1964).

The counter-current extraction system greatly amplifies the advantages of multiple stage extraction. We have used an all-glass device of this type for 5 to 20 gallon volumes of waste and it is highly efficient. However, it is fragile and requires a good deal of space, solvent, and attention.

IV MISCELLANEOUS METHODS

Recent work in our laboratories has shown that reverse-phase partition columns are quite suitable for extracting fairly large volumes of water provided that the solute and the organic solvent are relatively insoluble in water.

The partition column uses a support such as silica or a diatomaceous earth to hold an aqueous (or other strongly polar) solvent. A non-polar solvent is then allowed to percolate slowly down the column while a continuous partition of the solute takes place between the phases. With the reverse-phase column, the stationary support is rendered hydrophobic with silicone, etc. and serves to hold the non-polar organic phase in place while the aqueous phase flows downward through it.

Another device for 10-20 liter quantities is a carboy containing the desired amounts of water and solvent. It can be rolled or shaken until equilibrated, then the solvent removed by syphoning. The difficulty, of course, is in obtaining equilibrium.

This outline was prepared by R. H. Burttschell, former Chemist, Waste Identification and Analysis Program, Advanced Waste Treatment Research Laboratory, R. A. Taft Water Research Center.

Descriptors: Organic Matter, Separation Techniques, Solubility, Solvent Extractions, Water Analysis

THIN-LAYER CHROMATOGRAPHY

I INTRODUCTION

A Historical

1 Adsorption chromatography was discovered by Tswett in 1903. Tswett found that plant pigments (e.g., chlorophylls) can be separated by filtering a solution of them in petroleum ether through a column of calcium carbonate. He noticed that yellow and green zones were formed in the column.

2 Adsorption chromatography was introduced in 1931 when the method was used for the preparative chemistry of the polyene pigments, for example the separation of carotene into its components.

3 Partition chromatography for hydrophilic compounds was introduced in 1941.

4 Thin-layer chromatography was introduced by Stahl in 1956. He described an ingenious and practicable device for preparing layers of silica gel about 250 microns thick with a plaster of paris binder.

5 Thin-layer chromatography has proven useful for the quantitative estimation of pesticides and for the "clean-up" of sample aliquots prior to the estimation of their components by gas chromatography.

B Theory

1 Thin-layer chromatography is an analytical technique which has proven useful for the separation of components in a given system and to evaluate the components in that system.

2 All of the techniques of chromatography are based upon the same simple principle. They involve a moving system of some type (liquid or gas) which is in equilibrium with a stationary phase. When the stationary phase is a solid and the forces acting between it and the mixture are adsorptive in nature, the technique is called adsorption chromatography.

3 When the stationary phase is a simple liquid or a liquid held on some type of support, the chromatography is considered to be partition chromatography.

4 Diagnostic or qualitative thin-layer chromatography is used to determine the number of components in a system.

5 Preparative thin-layer chromatography is used to separate a mixture into its components in such a way that reasonable amounts can be isolated and studied.

6 Quantitative thin-layer chromatography is used to measure the amount of the components present. This is done by estimating the density of the spots as compared to standard spots.

II DEFINITION OF TERMS

A Adsorbent. The finely divided powder which makes up the stationary phase in adsorption chromatography, or holds the stationary liquid in partition chromatography.

B Layer. The thin layer of adsorbent bound or unbound, deposited on a glass plate.

C Spotting. The application of the substance to the thin layer adsorbent.

D Development. The passing of a liquid through the layer to effect a separation.

E Developer. The liquid used for the development.

F Eluent. The complete removal of a substance from the adsorbent layer.

G Visualization. The rendering visible of the results of a developed chromatogram.

H Ratio facter "R_f". The distance traveled by a given substance divided by the distance traveled by the solvent front. Both measured as the origin.

III ADSORBENTS

A Silicic Acid or Silica Gel

Chemically identical, but the gel is prepared in such a manner as to enhance its adsorptive power.

B Aluminum Oxide or Alumina

C Diatomaceous Earth

D Powdered Cellulose

E Essentially any substance which has desirable adsorptive and chemical characteristics can be successfully used in thin-layer chromatography.

IV PREPARATION OF PLATES

A Methods

1 Spreading or spraying of slurries

2 Dipping in a slurry

B Equipment

1 Kirchner type

2 Stahl type

3 Microchromatoplates

C Layer thickness

1 For qualitative analysis thin layers (250 microns) are used.

2 For preparative thin layer thicker layers may be used.

D Moisture Content

1 Trace quantities of water greatly reduce the activity of various adsorbents.

2 Normally plates are activated by drying at 110°C for one hour and then stored in a desiccator.

V TECHNIQUE

A Spotting the Plate

1 The activity of the layer is affected by humidity and temperature.

2 The sample is applied so that the direction of the development is opposite to the direction in which the layer has been applied.

3 Use of spotting template

4 Use of micro-pipette and micro-liter syringe

5 Size of sample spots

6 Use of standards

B Solvent System

1 Investigation of single and multi-component systems

2 Carbon tetrachloride, hexane, and n-heptane as solvents for separation of pesticides

C Development of the Plate

1 Development tank

2 The plate is placed in a glass tank containing the solvent. When the solvent front reaches the upper reference line (10 cm) the plate is removed and the solvent is allowed to evaporate.

VI VISUALIZATION

A Rhodamine B

1 Spray reagents - Rhodamine B, spirit soluble, 0.1 mg per ml in ethanol

2 Method of spraying

3 Advantages of Rhodamine B

a The pesticide is not destroyed.

b The exact position of the pesticide can be determined.

c The use of a selective solvent permits the pesticide to be eluted from the adsorbent while the Rhodamine B is retained.

B Impregnation of Plates with $AgNO_3$

1 Increased sensitivity for quantitative estimation

2 Elimination of spraying

C Exposure to UV Light

1 Pesticides appear as quenched areas in the fluorescent background.

2 Recovery studies using Rhodamine B spray are comparable to those using silver nitrate spray. The following pesticides may be seen at the 10 μg level under UV light after Rhodamine B spray:

endrin
dieldrin
DDT
aldrin
lindane
chlordane
parathion
methyl parathion
heptachlor
heptachlor epoxide
methoxychlor
toxaphene
DDE
ovex
tedion

D Suggested systems for adsorption chromatography are given in Table 1.

E Recovery

1 Elution of pesticides from the thin layer with a mixture of ethyl and petroleum ethers.

2 Recovery zones as shown in Figure 1.

3 Silica gel collection assembly - Figure 2.

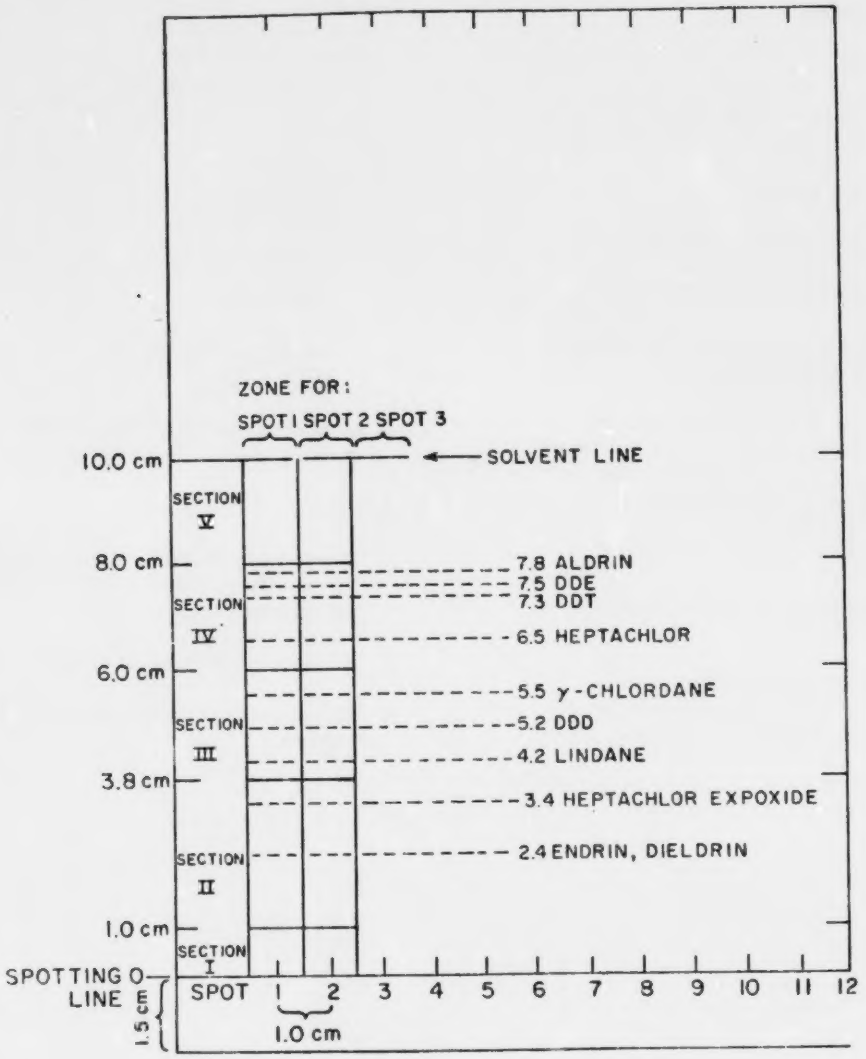
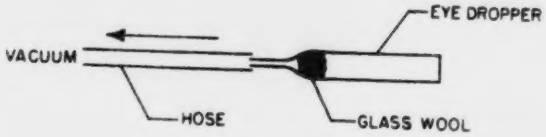

ZONE FOR:
SPOT 1 SPOT 2 SPOT 3
SOLVENT LINE
10.0 cm
SECTION V
8.0 cm
7.8 ALDRIN
7.5 DDE
7.3 DDT
SECTION IV
6.5 HEPTACHLOR
6.0 cm
5.5 γ-CHLORDANE
SECTION III
5.2 DDD
4.2 LINDANE
3.8 cm
3.4 HEPTACHLOR EXPOXIDE
SECTION II
2.4 ENDRIN, DIELDRIN
1.0 cm
SECTION I
SPOTTING LINE 0
1.5 cm
SPOT 1 2 3 4 5 6 7 8 9 10 11 12
1.0 cm

Figure 1. Diagram of Designation of Sections in the Cleanup and Separation of CCE-Aromatics on Silica Gel Layers.


EYE DROPPER
VACUUM
HOSE
GLASS WOOL

SILICA GEL COLLECTION ASSEMBLY

FIGURE 2

TABLE 1

SUGGESTED SYSTEMS FOR ADSORPTION CHROMATOGRAPHY

Compounds	Adsorbent	Developer	Visualization
1. Long-chain (over eight carbons) aliphatic ketones	silica gel G	a. benzene-EtOEt mixtures b. toluene-EtOEt mixtures c. pet. ether-EtOEt mixtures	phosphomolybdic acid (2, 4-DNPH)
2. Aliphatic and aromatic aldehydes and ketones	aluminum oxide	a. benzene b. benzene-EtOH (98:2) (95:5) (90:10) c. $CHCl_3$ d. EtOEt e. pet. ether- benzene (1:1)	2, 4-DNPH
3. Aromatic aldehydes and ketones	silica gel G	hexane-EtOAc (4:1) (3:2)	(U.V. on phosphors)
4. Vanillin and other substituted benzaldehydes	silica gel G	a. pet. ether-EtOAc (2:1) b. hexane-EtOAc (5:2) c. $CHCl_3$-EtOAc (98:2) d. decalin-CH_2Cl_2-MeOH (5:4:1)	(U.V. on phosphors)
5. 2, 4-Dinitrophenylhydrazones of aldehydes	silica gel G	a. benzene-pet. ether (3:1) for aliphatic b. benzene-EtOAc (95:5) for aromatic	colored
6. Miscellaneous alkaloids	silica gel G	a. $CHCl_3$-acetone-diethylamine (5:4:1) b. $CHCl_3$-diethylamine (9:1) c. cyclohexane-$CHCl_3$-diethylamine (5:4:1) d. cyclohexane-diethylamine (9:1) e. benzene-EtOAc-diethylamine (7:2:1)	(Dragendorff's Reagent)
	aluminum oxide	f. $CHCl_3$ g. cyclohexane-$CHCl_3$ (3:7) plus 0.05% diethylamine	
7. Alkaloids and barbiturates in toxicology	silica gel G	a. MeOH b. $CHCl_3$-EtOEt (85:15)	(Dragendorff's Reagent)
8. Strong-base amines	unbound aluminum oxide	a. acetone-heptane (1:1) b. $CHCl_3/NH_3$ (sat. at 22°)-EtOH (96%) (30:1)	I_2 vapor-U.V. (Dragendorff's Reagent)
9. Sugar acetates and inositol acetates	silica gel G	benzene with 2-10% MeOH	H_2O

TABLE 1 (continued)

SUGGESTED SYSTEMS FOR ADSORPTION CHROMATOGRAPHY

Compounds	Adsorbent	Developer	Visualization
10. Aldose 2,4-DNPH's	a. aluminum oxide G b. silica gel G	a. toluene-EtOAc (1:1) b. toluene-EtOAc (3:1) (1:1)	a. colored compounds b. NaOH
11. Cardiac glycosides	silica gel G	a. $CHCl_3$-MeOH (9:1) b. $CHCl_3$-acetone (65:35)	H_2SO_4 and heat
12. Dicarboxylic acids	silica gel G	a. benzene-MeOH-HOAc (45:8:4) b. benzene-dioxane-HOAc (90:25:4)	bromphenol blue acidified with citric acid
13. p-Hydroxybenzoic acid esters	silica gel G	pentane-HOAc (88:12)	(U.V. on phosphors)
14. Sulfonamides	silica gel G	$CHCl_3$-EtOH- heptane (1:1:1)	p-dimethylamino-benzaldehyde, acidified (U.V. on phosphors)
15. Food dyes	silica gel G	a. $CHCl_3$-acetic anhydride (75:2) b. benzene c. methyl ethyl ketone-HOAc-MeOH (40:5:5)	$SbCl_3$ in $CHCl_3$
16. Misc. essential oils	silica gel G	benzene-$CHCl_3$ (1:1)	$SbCl_3$ in $CHCl_3$
17. Coumarins	silica gel G	a. pet. ether-EtOAc (2:1) b. hexane-EtOAc (5:2)	(U.V. on phosphors)
18. Alkali metals-Na^{1+}, Li^{1+}, K^{1+}, Mg^{2+}	purified silica gel G	EtOH-HOAc (100:1)	acid violet (1.5% aqueous soln.)
19. 24 ferrocene	silica gel G	a. benzene b. benzene-EtOH (30:1) (15:1) c. propylene glycol-MeOH (1:1) d. propylene glycol-chlorobenzene-MeOH (1:1:1)	(U.V. on phosphors)
20. Misc. lipids	silica gel G	a. EtOEt b. isopropyl ether c. isopropyl ether-HOAc (98.5:1.5)	(H_2SO_4 and heat)
21. Fatty acid methyl esters	silica gel G	hexane-EtOEt mixtures (up to 30% EtOEt)	a. I_2 b. H_2SO_4 and heat
22. Glycerides	silica gel G	a. Skellysolve F-EtOEt (70:30)	H_2SO_4 and heat

TABLE 1 (continued)

SUGGESTED SYSTEMS FOR ADSORPTION CHROMATOGRAPHY

Compounds	Adsorbent	Developer	Visualization
		b. (10:90) for monoglycerides c. (70:30) for diglycerides d. (90:10) for triglycerides e. (60:40) (35:65) (85:15)	
23. Long-chain aliphatic alcohols	silica gel G	a. pet. ether-EtOEt-HOAc (90:10:1) b. pet. ether-EtOEt (20:80) (10:90) (70:30)	H_2SO_4 and heat
24. Phenols	a. starch-bound silicic acid kieselguhr (1:1)	a. xylene b. xylene-$CHCl_3$ (3:1) (1:1) (1:3) c. $CHCl_3$	(U.V. on phosphors)
25. Phenols and phenolic acids	starch-bound silicic acid with phosphors	a. Skellysolve B- EtOEt (3:7) b. Skellysolve B- EtOAc (1:3) c. Skellysolve B-acetone (3:1)	(U.V. on phosphors)
26. Phenols	plaster-of-Paris bound silica gel	a. hexane-EtOAc (4:1) (3:2) b. benzene-EtOEt (4:1) c. benzene	(U.V. on phosphors)
27. 3, 5-Dinitrobenzoates of alcohols and phenols	silica gel G	a. benzene-pet. ether (1:1) b. hexane-EtOAc (85:15) (75:25) c. toluene-EtOAc (90:10)	colored compounds
28. Steroids	silica gel G	a. benzene b. benzene-EtOAc (9:1 (2:1) c. cyclohexane-EtOAc (9:1) (19:1) d. 1, 2-dichloroethane	$SbCl_3$ in $CHCl_3$
29. 19-Nor-steroids	silica gel G	EtOAc-cyclohexane mixtures	$SbCl_3$ in $CHCl_3$
30. Blood cholesterol and cholesterol esters	silica gel G	a. benzene b. benzene-EtOAc (9:1) c. 1, 2-dichloroethane d. $CHCl_3$	$SbCl_3$ in $CHCl_3$
31. Triterpenoids	silica gel G	a. isopropyl ether-acetone (5:2) (19:1) b. isopropyl ether c. cyclohexane d. benzene e. CH_2Cl_2	(H_2SO_4 and heat)

TABLE 1 (continued)

SUGGESTED SYSTEMS FOR ADSORPTION CHROMATOGRAPHY

Compounds	Adsorbent	Developer	Visualization
32. Carotenes and fat-soluble vitamins, A, D, E, K	unbound aluminum oxide	a. MeOH b. CCl_4 c. xylene	H_2SO_4 and heat
33. Thiophene derivatives	a. silica gel G b. aluminum oxide G	a. benzene-$CHCl_3$ (9:1) b. MeOH c. pet. ether	(U.V. on phosphors)
34. Plasticizers (phthalates, phosphates and other esters)	silica gel G with phosphors	a. isooctane-EtOAc (90:10) b. benzene-EtOAc (95:5) c. butyl ether - hexane (80:20)	a. (U.V. on phosphors) b. I_2

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This outline was prepared by P. F. Hallbach, Chemist, National Training Center, Water Programs Operations, EPA, Cincinnati, OH 45268.

Descriptors: Adsrobents, Chromatography, Organic Matter, Separation Techniques, Water Analysis

INTRODUCTION TO GAS-LIQUID CHROMATOGRAPHY

Part I

I INTRODUCTION

A Definition

Gas-liquid chromatography is an analytical method for the separation and identification of a mixture of volatile (usually organic) components in a sample. As with any chromatographic technique the column consists of two phases, the immobile or stationary phase (a liquid on an inert solid support), and the mobile phase (an inert gas). The column functions to separate the sample components because they have varying vapor pressures and affinities for the stationary phase. In many ways the column behavior resembles that of fractional distillation. The partition which occurs between the mobile and immobile phases will thus cause the components to proceed through the column at varying rates. The separation is recorded and quantitated by the detector system.

B Advantages

1 GLC can be used to separate compounds of similar boiling points which cannot easily be separated by distillation. (See Table 1)

2 GLC can be extremely sensitive; for example, using the electron capture detector it is possible to "see" picogram (10^{-12}) quantities.

Table 1. SEPARATIONS BY GLC

Compounds	Reference
3-Methylcyclohexene (B.P. 104° C) and 4-Methylcyclohexan (B.P. 103° C)	Aerograph Research Notes (Spring 1964)
Cyclohexane (B.P. 80.8° C) and Benzene (B.P. 80.2° C)	Chromosorb Newsletter (FF-104)

C Disadvantages

1 Due to the extreme sensitivity possible it is often necessary to apply extensive cleanup techniques.

2 The many variables of the technique require a skilled analyst.

II COMPONENTS OF A GAS CHROMATOGRAPH (See Figure 1)

A Gas Supply

The mobile phase (carrier gas) transports the sample components through the column into the detector. The type of gas used varies with the detector.(See Table 2)

B Injector

Liquid samples are manually introduced into the heated injector block through a rubber septum by means of a syringe. Automatic liquid injectors as well as injection systems for solid and gaseous samples are commercially available.

C Column

The vaporized sample enters the column which can be glass or metal and of varying length (1' - 20') and diameter (1/8" - 1/4"). The column is packed with the stationary (immobile) phase and contained within a constant temperature oven.

1 Solid support

The solid support should have a large surface area yet be inert so that active sites will not cause adsorption of sample components. Diatomaceous earths, teflon and glass beads have been used. (See Table 3)

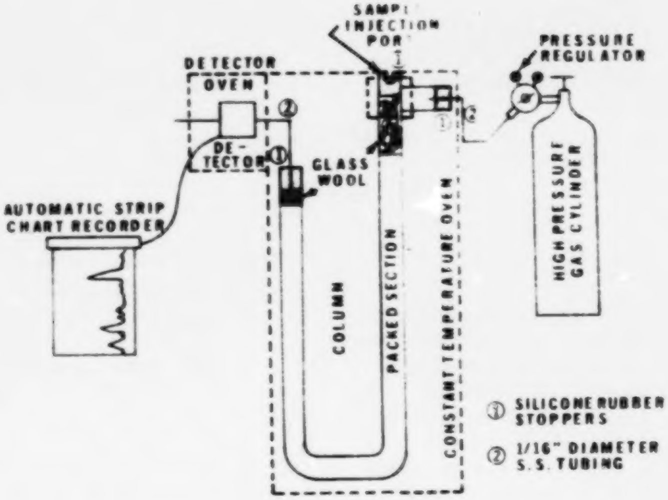
SAMPLE INJECTION PORT
PRESSURE REGULATOR
DETECTOR OVEN
②
DE-TECTOR
①
GLASS WOOL
①
②
AUTOMATIC STRIP CHART RECORDER
HIGH PRESSURE GAS CYLINDER
COLUMN
PACKED SECTION
CONSTANT TEMPERATURE OVEN
① SILICONE RUBBER STOPPERS
② 1/16" DIAMETER S.S. TUBING

Figure 1. COMPONENTS OF A GAS CHROMATOGRAPH*

Table 2. CARRIER GASES

Detector	Carrier gas
Thermal conductivity	Helium (Purified, Grade A)
Microcoulometric	Helium (Purified, Grade A)
Flame ionization	Hydrogen (Purified)
Electron capture	Nitrogen or a mixture of 95% argon and 5% methane (Purified)

Table 3. SOLID SUPPORTS

Support	Surface area (m^2/gm)
Chromosorb P (Diatomaceous Earth)	4.8
Chromosorb W (Diatomaceous Earth)	1.2
Chromosorb G (Diatomaceous Earth)	0.5
Chromosorb T (Teflon)	7.0 - 8.0

2 Stationary liquid

The separation and partition occurring in the column is directly affected by the choice of stationary liquid. For example, in the separation of benzene (B. P. 80.1°C) and cyclohexane (B. P. 80.8°C), the choice of a non-polar phase such as hexadecane results in benzene preceding cyclohexane off the column. However, if a more polar phase such as benzylbiphenyl is chosen cyclohexane precedes benzene. Table 4 shows some typical stationary liquids and their uses. (NOTE: One requirement for any liquid is that it have a high boiling point so that it will not boil off the column)

D Detector

The detector or brain of the gas chromatograph senses and measures the quantity of sample component coming off the column. The detector should be maintained at a temperature higher than the column so that condensation does not occur in the detector block. Several types of detectors are in use today.

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*Reproduced (with permission) from Chemistry. (37:11, p. 13. November 1964).

Table 4. STATIONARY LIQUIDS

Stationary liquid	Used to separate
Silicone oils QF-1, Dow Corning 200, and Dow 11, OV-1, OV-3, OV-17, OV-Z10, OV-225	Chlorinated hydrocarbons pesticides
Silicone oil SE-30	Homologous series of n-alkanes
Benzyl-Cyanide-Silver Nitrate	Homologous series of olefins
Polyethylene Glycol	Amines
Cyano Silicone	Steroids

1 Thermal conductivity

This detector consists of a Wheatstone bridge two arms of which are thermal conductivity cells each containing a small heated element. When only carrier gas is flowing through both the sample cell and reference cell, the resistance of the heated element is constant in both cells. The bridge remains balanced and baseline is recorded. However, when carrier gas plus sample component enter the sample cell, the thermal conductivity in that cell changes thus also producing a change in the resistance of the heated element. The bridge becomes unbalanced and a peak is recorded. The main disadvantage of the TC cell in water pollution work is its lack of sensitivity.

2 Ionization detectors

a Flame

This detector consists of a flame situated between a cathode and anode. As carrier gas alone burns, some electrons and negative ions are produced which are collected at the anode and recorded as baseline. When carrier gas plus sample component are burned, more electrons and negative ions are produced which result in a peak on the recorder. The detector is capable of "seeing" nanogram quantities of organic compounds; however, the detector is sensitive to all organic compounds. This lack of specificity produces disadvantages in the analysis of water extracts which contain a variety of naturally occurring organics.

b Electron capture (See Figure 2)

This detector consists of a radiation source (e.g., tritium) capable of producing slow electrons in a carrier gas such as nitrogen. The electrons collected at the anode are recorded as baseline. When sample components which have an electron affinity (e.g., chlorinated hydrocarbons) enter the detector, electrons are "captured." The subsequent decrease in current is recorded as a peak. The detector has the advantage that it is extremely sensitive (picogram range) and is somewhat selective.

c Thermionic

A recent adaptation of the flame ionization detector shows promise for the specific analysis of organic phosphorus compounds. An alkali salt is incorporated into the design of a conventional flame ionization detector so that the salt heated by the flame produces an ion current. When compounds containing phosphorus emerge from the column, they give 600X the response with this detector as with the conventional flame.

3 Microcoulometric

Although less sensitive (by approximately a factor of 10) than electron capture,

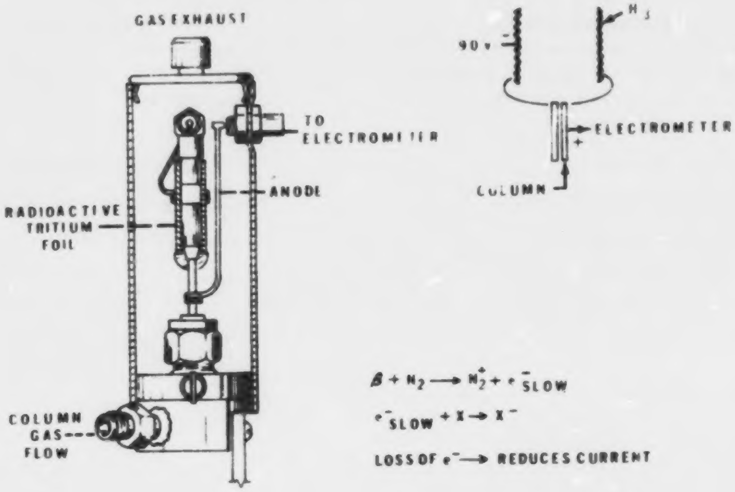
GAS EXHAUST
TO ELECTROMETER
ANODE
RADIOACTIVE TRITIUM FOIL
COLUMN GAS FLOW
90 v
H_3
ELECTROMETER
COLUMN
$\beta + N_2 \longrightarrow N_2^+ + e^-_{SLOW}$
$e^-_{SLOW} + X \rightarrow X^-$
LOSS OF e^- → REDUCES CURRENT

Figure 2. ELECTRON CAPTURE DETECTOR (Wilkens Instrument Company)

this detector is finding wide use in pesticide analysis. This highly specific detector consists of titration cells for the measurement of chloride-containing and sulfur-containing compounds. The sample component emerging from the column is combusted to produce HCl or SO_2, respectively. HCl is continuously titrated by silver ions present in the cell; the amount of current required to regenerate these silver ions is recorded as a peak. The system for sulfur containing compounds is analogous except that SO_2 produced is continuously titrated by I_2 which is subsequently regenerated.

Another microcoulometric detector has recently been applied to the specific determination of nitrogen. It is based on the reduction of nitrogen-containing compounds to NH_3 which is then titrated by H^+ in the titration cell.

4 Coulson Electrolytic Conductivity

This detector was primarily developed for the detection of organic halides, organic nitrogen compounds, and organic sulfur compounds.

The unit consists of pyrolyzer with a separately heated inlet block, water circulating and purification system, detector cell and dc conductivity bridge. The sample is oxidized or reduced and reaction products form electrolytes when dissolved in the deionized water. Changes in conductivity between two platinum electrodes are measured by the dc bridge. (Figure 2 a)

E Recorder

The recorder system registers the response of the detector to sample components. In the case of ionization detectors, it is often necessary to employ an electrometer in order to amplify the small current changes.

Expensive integration and digital read-out equipment is also available to facilitate measurement of peak areas.

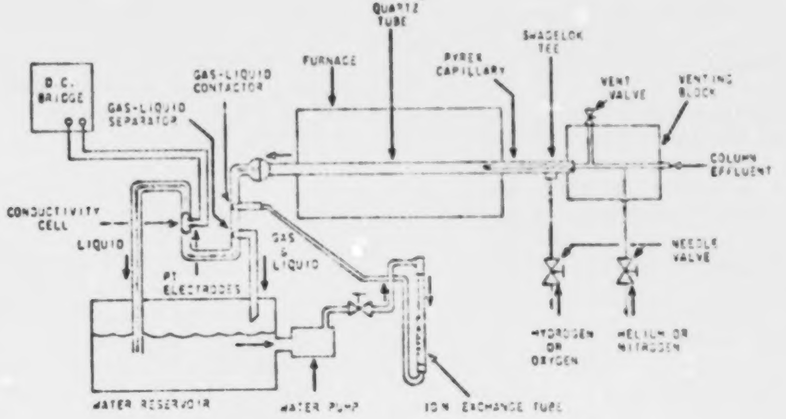
QUARTZ TUBE
D.C. BRIDGE
GAS-LIQUID CONTACTOR
FURNACE
PYREX CAPILLARY
SWAGELOK TEE
VENT VALVE
VENTING BLOCK
GAS-LIQUID SEPARATOR
COLUMN EFFLUENT
CONDUCTIVITY CELL
LIQUID
GAS & LIQUID
PT ELECTRODES
NEEDLE VALVE
HYDROGEN OR OXYGEN
HELIUM OR NITROGEN
WATER RESERVOIR
WATER PUMP
ION EXCHANGE TUBE

Figure 2 a. FLOW DIAGRAM OF ELECTROLYTIC CONDUCTIVITY DETECTOR (Coulson Instruments Company)

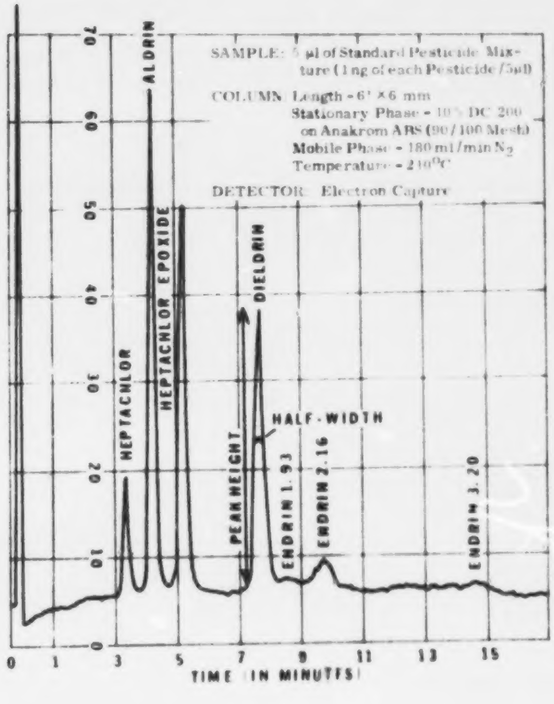

Figure 3. GAS CHROMATOGRAM OF PESTICIDE MIXTURE

III QUALITATIVE ANALYSIS

A Retention Time

The retention time of a sample component is defined as the time it takes for that component to travel through the column. There are a number of variables which affect the retention time of a compound.

1 Physical parameters of column operation
   a Column length
   b Column temperature
   c Carrier gas flow rate
2 The nature and amount of stationary liquid itself

For a given set of column conditions, a specific compound will have a specific retention time (See Figure 3 and Table 5). Various column and detector combinations can be used to confirm identification.

B Retention Volume

Retention volume is defined as the total volume of gas required to move a component through the column.

$$\text{RETENTION VOLUME } (R_V) = \text{RETENTION TIME } (R_T) \times \text{FLOW RATE}$$

C Relative Retention Times and Volumes

It is possible to interpret data more easily by reporting retention data relative to a particular compound (e.g., aldrin as in Table 5).

IV QUANTITATIVE ANALYSIS

A Measurement of Peak Area

The quantity of sample component present is directly proportional to the area under its peak. (NOTE: This assumption can only be made if it has been previously determined that a linear response is obtained in the range under study.) The following are a few of the ways in which this area can be measured.

1 Planimeter
2 Triangulation
3 Peak height × half-width (see dieldrin peak in Figure 3)

AREA = peak height × peak half-width

4 Disc integrator

B Measurement of Peak Height

With the electron capture detector it may be possible to use peak height for quantitative measurements where the following conditions are met.

Table 5. RETENTION DATA FOR FIGURE 3

Pesticide	Retention Time (R_T)	Relative Retention Time
Heptachlor	3.3 minutes	0.79
Aldrin	4.2	1.00
Heptachlor Epoxide	5.3	1.26
Dieldrin	7.7	1.84

1 A steady basline is obtained.

2 Retention times can be reproduced from one injection to the next.

V SUMMARY

The basic components of a gas chromatograph have been described. Elementary aspects of quantitative and qualitative analysis are presented.

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This outline was prepared by B. A. Punghorst, Chemist, formerly with National Training Center, MDS, WPO EPA, Cincinnati, OH 45268.

Descriptors: Adsorbents, Chromatography, Gas Chromatography, Organic Matter, Separation Techniques, Water Analysis

BASIC THEORY OF GAS-LIQUID CHROMATOGRAPHY

(PART II)

Gas chromatography is a method for separating the components of a mixture of compounds of sufficient volatility to be varporized. It is usually used to identify and quantitate the components in a mixture; it also is used to prepare small amounts of extremely pure materials. For this reason it is frequently used in conjunction with mass spectrometry, infrared spectroscopy, or NMR spectroscopy.

In chromatography the stationary phase may be a gas, liquid, or a solid, and the mobile phase likewise. The various possible combinations are shown in Table 1.

Table 1.

		Stationary Phase		
		gas	liquid	solid
Mobile Phase	gas	---	GC	GS
	liquid	---	LL	LS
	solid	---	---	zone refining

The apparatus used consists of a long, thin column packed with a solid support, such as firebrick crushed and screened, coated with a liquid phase, such as stopcock grease, through which is passing a carrier gas, frequently helium or nitrogen. Ahead of the column is the injector, through which the sample is introduced, and at the exit is the detector, which produces an electrical signal when the sample is emerging from the column. This signal is fed to a strip chart recorder. The heart of the system is the column, the part in which the separation takes place.

Henry's law states that a solute will distribute itself between the gas and liquid phases. That is, an equilibrium is established in which part of the solute is in the liquid phase and part is in the gas phase. This condition is defined by the Henry's Law Constant, or

$$\text{Partition Coefficient, } K = \frac{\text{amt. in liquid phase}}{\text{amt. in gas phase}}.$$

Different substances have different partition coefficients. A substance will only move down the column when it is in the gas phase. Thus a substance which spends the largest percentage of its time in the gas phase will come out of the column, or elute, first.

Imagine that a column is made up of a series of buckets partially filled with a stationary liquid phase. A unit amount of a substance to be separated is introduced into the first bucket and is allowed to distribute itself between the liquid and gas phases within the bucket until an equilibrium has been established. Imagine that the substance in the gas phase is then swept into a second bucket by a carrier gas and that both buckets are allowed to come to a new equilibrium.

This process is repeated with a 3rd, 4th, and 5th bucket as illustrated in Figure 1. The column can be considered as consisting of thousands of these buckets. It is seen that the substance distributes itself along the column as a Gaussian function and moves along the column as a function of time.

In an ideal column the profile would be as shown in Figure 2, with the material in the gas phase moving down the column and entering the liquid phase at the leading edge while it is coming from the liquid into the gas phase at the trailing edge.

Equilibrium 1.

1/2 1/2

Step 1.

1/2	1/2	

Equilibrium 2.

1/4 1/4	1/4 1/4

Step 2.

1/4	1/4	1/4	1/4	

Equilibrium 3.

1/8 1/8	1/4 1/4	1/8 1/8

Step 3.

1/8	1/8	1/4	1/4	1/8	1/8	

Equilibrium 4.

1/16 1/16	3/16 3/16	3/16 3/16	1/16 1/16

Step 4.

1/16	1/16	3/16	3/16	3/16	3/16	1/16	1/16	

Equilibrium 5.

1/32 1/32	1/8 1/8	3/16 3/16	1/8 1/8	1/32 1/32

Figure 1. DISTRIBUTION OF A SUBSTANCE ALONG A GAS CHROMATOGRAPHIC COLUMN

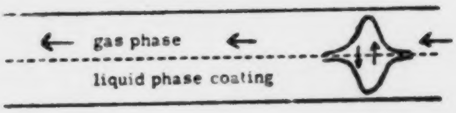

Figure 2

Since separation takes place because of differences in K, the stationary liquid phase should dissolve all components to some extent, but no two to exactly the same extent. Since these differences may be very small, columns are usually of some length and must be very efficient. The same argument follows when solid adsorbants are used instead of liquid phases, as in gas-solid chromatography.

Efficiency is the ability to separate two substances and is defined in several ways. Let us examine a typical simple chromatogram and define some terms. Figure 3 shows the separation of a mixture of cyclohexane, benzene, and toluene. The point of injection is marked "start". This is followed by the air peak, which is a measure of the dead volume of the column. By drawing tangents to the peaks we determine the center. The distance from start to the center of the peak is the retention time, since the recorder is running at constant speed, and is designated by t_R. The peak width, w, is the intersection of the tangents with the base line.

The adjusted retention time, t_R, is the retention time minus the distance from the start to the air peak. The retention volume, V_R, is the retention time, t_R, times the carrier gas flow rate, F_c. Likewise, the adjusted retention volume $V'_R = t'_R F_c$. We speak of the number of plates, n, in a column. This is calculated from the expression, $n = 16\frac{(t_R)}{w}$. The height equivalent to a theoretical plate, HETP, is the column length in mm divided by the number of plates, n. For Benzene, in Figure 3, $w_1 = 9$ mm, $t_1 = 56$ mm, $n = 16(\frac{56}{9})^2 = 620$ plates. Note that the dimensions cancel. HETP $= \frac{5 \times 12 \times 25}{620}$ mm $=$ 2.4 mm. It is the HETP, or plate height that is commonly used to express column performance.

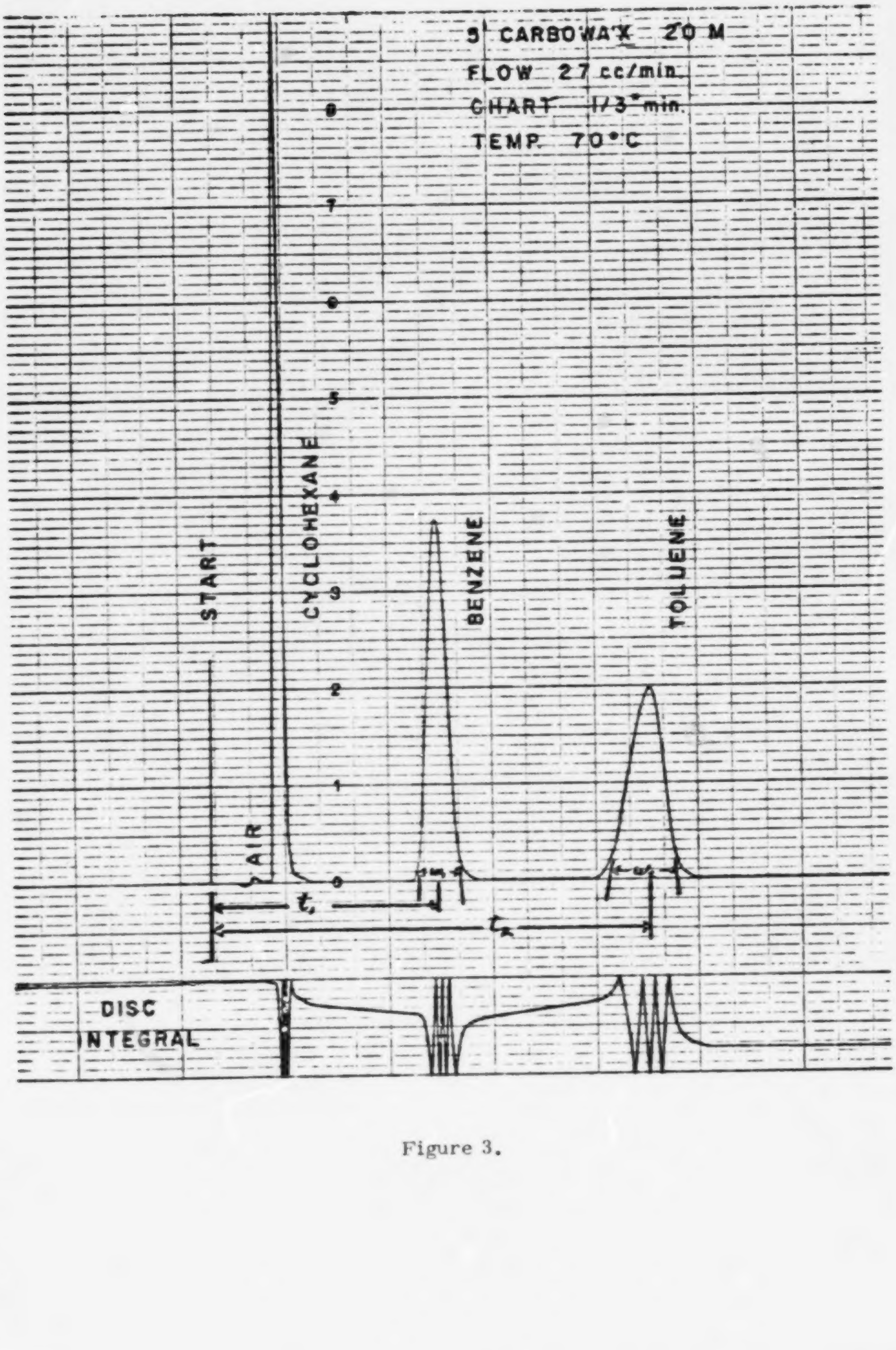
5' CARBOWAX 20 M
FLOW 27 cc/min
CHART 1/3"/min
TEMP 70°C
8
7
6
5
4
3
2
1
0
START
AIR
CYCLOHEXANE
BENZENE
TOLUENE
t_1
t_2
DISC
INTEGRAL

Figure 3.

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Kaiser, R. (translated by P.H. Scott). Gas Phase Chromatography. Butterworths, Washington, 1963. This work is in three volumes and is very concise and complete. Volume III has a great deal of retention data.

Volume I	Gas Chromatography
Volume II	Capillary Chromatography
Volume III	Tables for Gas Chromatography

Collected Papers from Symposia held by Gas Chromatography Discussion Groups. These are published under the title "Gas Chromatography 19__." The even years, which were held in Europe, are published by Butterworths, Washington, D.C.; the odd years, which were sponsored in this country by ISA, are published by Academic Press, New York. The editions are edited by:

1957 - Coates, V.J. et al.
1958 - Desty, D.H.
1959 - Noebels, H.J. et al.
1960 - Scott, R.P.W.
1961 - Brenner, N. et al.
1962 - van Swaay, M.
1963 - Fowler, L.

Though these books are a series of papers, many of the basic advances in the state of the art were reported at these meetings. The results of discussions which followed the papers are also included.

Journals

A good share of all papers dealing with gas chromatography are published in three journals, Analytical Chemistry, Journal of Chromatography, and Journal of Gas Chromatography. Some of the earlier articles appeared in Nature.

Abstracts

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"Chromatogram", Barber Colman Company, Rockford, Illinois.

"Instrument News", the Perkin-Elmer Company, Norwalk, Connecticut.

This outline was prepared by J.W.Amy, Professor of Chemistry, Purdue University, Lafayette, Indiana.

Descriptors: Adsorbents, Chromatography, Gas Chromatography, Organic Matter, Separation Techniques, Water Analysis

THEORY OF GAS CHROMATOGRAPHY PEAK BROADENING

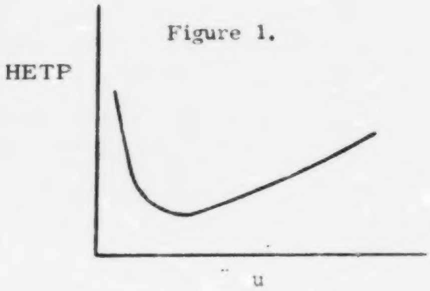
(PART III)

I THEORY

The equation which expresses the contribution of column performance to plate height was first proposed by van Deemter and bears his name. In the simple form it is:

$HETP = A + \frac{B}{u} + Cu$ where u is the linear gas velocity and A, B, and C are constants for a given column. A plot of HETP vs. u, called a van Deemter plot, (Figure 1) is frequently used to establish proper flow rates.

Figure 1.



van Deemter plot

This expression, though not precisely correct, is useful because it gives a concise description of efficiency as a function of flow rate and can be used to eliminate flow as a variable and discuss other column functions in terms of A, B, and C. The A term, which is also given as $A = 2\lambda d_p$, or a constant times the particle diameter, is the limiting case when u is zero. The constant λ describes the dispersion of sample crossing a particle diameter and has a value of about 2 for common columns. The B term, often called the gas term, describes the diffusion of the solute in the gas phase and is a function of the vicsocity of the gas and the diffusivity of the solute. C is a term which describes the slowness of mass transfer between the liquid and gas phases, and is an acknowledgment that this equilibrium is not instantaneous. Purnell, Golay, Giddings and others have pointed out more exact expressions and modifications of the van Deemter equation. These are especially needed in capillary and highly efficient packed columns. However, the simple form is quite adequate for understanding the factors involved and describes normal packed columns very well.

II SOURCES OF PEAK BROADENING

There are many sources contributing to peak broadening in a chromatographic system. These can be divided into two classes; those due to faults in the instrument and those due to the column.

A Instrumental Broadening

1 Injector - The purpose of the injector is to vaporize the sample and introduce it into the carrier gas as a slug. Poor injection may be due to insufficient heat to vaporize the sample, excessive volume in the injector, or poor sweeping of the chamber by the carrier gas.

2 Fittings - Connections and fittings frequently have high dead volumes and abrupt changes in size which cause excessive mixing and peak broadening.

3 Detector - Detection systems with high volumes can cause peak broadening.

4 Non-uniform temperature in the column - ovens, especially long ones, often have large temperature gradients in them. As a result the various parts of the column are at different temperatures, hence operating under different conditions.

E Column Parameters

1 Natural band width - In the development of the plate concept we arrived at a Gaussian shaped curve. This is the minimum width for an isothermal chromatogram in the ideal case.

2 Longitudinal diffusion - This is the "B" term in the van Deemter equation and takes place almost entirely in the gas phase. The peak broadens, remains symmetrical.

3 Non-instantaneous vapor equilibrium - This is the van Deemter "C" term and broadens peaks because of the finite time needed for mass transfer from the gas to the liquid phase. This results in sharp fronts and tailing backs.

4 Packing geometry - The packing in the column is frequently not uniform and thus we have non-uniform gas flow. The smaller particles tend to go to the column center, the large ones to the wall. Also "race tract" effects due to tightly coiled large columns frequently result in tailing fronts, sharp backs.

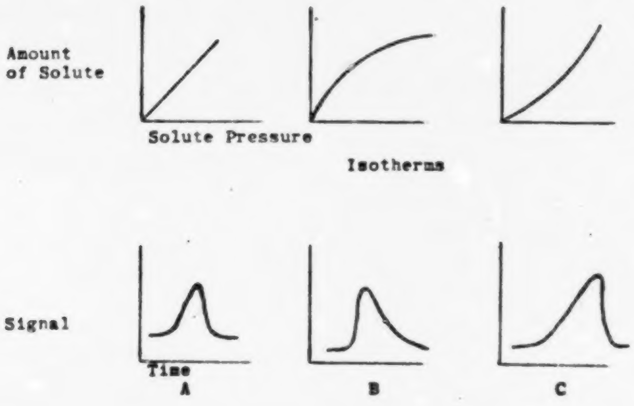
5 Non-linear isotherms - The ideal case assumes that a plot of solute amount vs. solute partial pressure is linear. Deviations may be either concave or convex. (Figure 2.)

6 Sample overload - When sample size exceeds the capacity of the column one gets into a non-linear portion of the isotherm. Condition 5-B is the most common with a sharp leading edge and a long tail.

7 Operating at a non-optimum linear velocity - This condition exists when operating outside the broad minimum of the van Deemter plot. When a column was a high pressure drip, only a portion of the column is operating at the optimum linear velocity.

8 Amount of liquid phase - Highly loaded columns are less efficient due to the higher value of the "C" term. However, low loading reduces capacity and hence sample size (6).

Figure 2.



Corresponding Chromatograms

9 Particle size and shape - The van Deemter A term. Particles should be uniform in size and shape; smaller sizes give more efficient columns until pressure drop becomes a limiting factor.

10 Column diameter - Efficiency decreases as column diameter increases because of non-uniform flow and temperature distribution. Special techniques such as mixing sections will help to overcome this.

11 Column length - The number of plates increases linearly with length until pressure drop becomes excessive.

12 Carrier gas - The optimum linear velocity changes with the carrier gas. However, this may be adjusted, and the choice of carrier is usually based on other criteria.

It can be seen that the final column design is always a series of compromises. The important factor is to have a system which will do the required analysis in the shortest possible time. Scott pictures the three related parameters, speed, resolution, and sample size, as being a triangle. (Figure 3.) Fixing any two will determine the third.

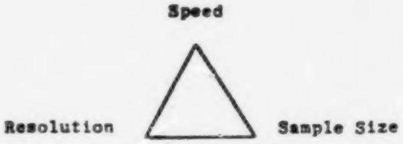


Figure 3.

This outline was prepared by J. W. Amy, Professor of Chemistry, Purdue University, Lafayette, Indiana.

Descriptors: Adsorbents, Chromatography, Gas Chromatography, Organic Matter, Separation Techniques, Water Analysis

CHARACTERISTICS OF ELECTRON CAPTURE AND OTHER IONIZATION DETECTION SYSTEMS

Any gas chromatographic detection system consists of a sensing element and various electrical amplifiers, attenuators, recorders and integrators used to measure and record the signal from this sensor. Some detection systems, such as the flame ionization detector, respond to some common molecular parameter and the response is known for many compounds. Other detectors, such as the electron capture detector, are very specific in their response to electronegative compounds and have virtually no response for other substances.

One family of detectors is based on measuring the change in electrical properties of gas conducting electricity. These are based on the principle that if a gas is made to conduct electricity, then the system is very sensitive to small changes in the composition of the gas. Such detector systems include:

A Argon ionization system which works on the basis of using a radioactive source to raise the argon carrier to a metastable state, then observe the ion current generated by the ionization of other molecules as the argon metastables decay.

B Cross section ionization which works on the basis of radioactive bombardment of the gas in a detector and measuring the ion current. Hydrogen is often used as a carrier, since it has the smallest cross section and hence is ionized to the least extent. Materials with higher cross sections will have much more chance of being ionized, and hence produce positive currents.

C "Helium" detector which is used for determining very low levels of materials in a helium matrix.

D Electron Capture Detector

1 Radioactive source, which ionizes nitrogen carrier gas, producing thermal ions and large current. When electronegative sample enters the cell, it "captures" some of these electrons before they reach the anode resulting in a decrease in ion current.

2 Photoionization source which produces thermal electrons which are captured as above.

E Flame emission detectors, in which the spectral energy of a substance, such as phosphorus or sulfur, is examined with a photomultiplier.

F Thermionic emission detectors in which an alkali metal, in or near the flame, changes its rate of volatization and hence the number of ions present when halogens or phosphorus are introduced into the flame.

This outline was prepared by J.W. Amy, Professor of Chemistry, Purdue University, Lafayette, Indiana.

Descriptors: Chemical Analysis, Chromatography, Gas Chromatography, Instrumentation, Water Analysis

IONIZATION DETECTORS

I General theory of operation

Ionization detectors operate on the principle that the electrical conductivity of a gas is directly proportional to the concentration of charged particles within the gas. Figure 1 shows a generalized ionization detector in which the ionizing source is unspecified.

Effluent gas from the column flows through the electrode gap past an ionizing source which ionizes some of the molecules in the gas stream. The presence of charge particles (positive ions, negative ions, electrons) within the electrode gap causes a current, I, to flow across the gap and through a measuring resistor R_2. The resulting voltage drop E_o across R_2 is amplified by an electrometer and fed into a recorder.

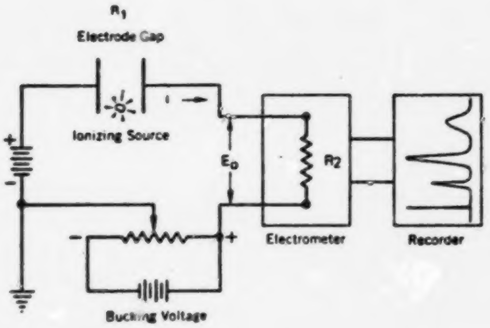


FIGURE 1 -SCHEMATIC IONIZATION DETECTOR CIRCUIT

It is helpful to think of the electrode gap as a variable resistor R_1 whose resistance value is determined by the number of charged particles within the gap. With pure carrier gas flowing a constant concentration of charged particles will be present in the gap, thus causing a constant current, I, to flow. This constant current is called the "background current." The background current is reduced to zero by opposing it with a "bucking voltage". Thus, under "no signal" conditions (pure carrier gas flow), no current flows, and the recorder traces a straight baseline. When a sample component passes through the electrode gap, molecules of the component are ionized. This increases the number of charged particles causing current to flow which produces a signal which is registered as a peak on the recorder.

II Flame ionization detector (5)

A Operating principle

Figure 2 shows a typical FID in which the effluent gas from the column is mixed with hydrogen and burned in air. Ions formed in the flame cause a current to flow in the external circuit. Sternberg et al (1) give a comprehensive discussion of flame ionization theories.

B Detector response

The FID responds to virtually all compounds with the exception of those listed in Table 3; note particularly the lack of response to air, H_2O and CS_2. The lack of response to air and water makes the FID especially suitable for the analysis of air pollutants or aqueous samples such as alcoholic beverages, biological materials, etc. Similarly, the absence of a "solvent peak" makes carbon disulfide a convenient solvent for use with the FID.

C Flow rate vs detector response

The FID performance depends on the proper choice of gas flow rates. Use a 1:1:10 relative flow ratio of hydrogen/carrier/air. Thus a carrier flow of 30 ml/min requires 30 ml/min of hydrogen and 300 ml/min of air for optimum performance of the FID. It may be necessary to use a wider orifice with a high flow rate in order to reduce the linear gas velocity through the burner.

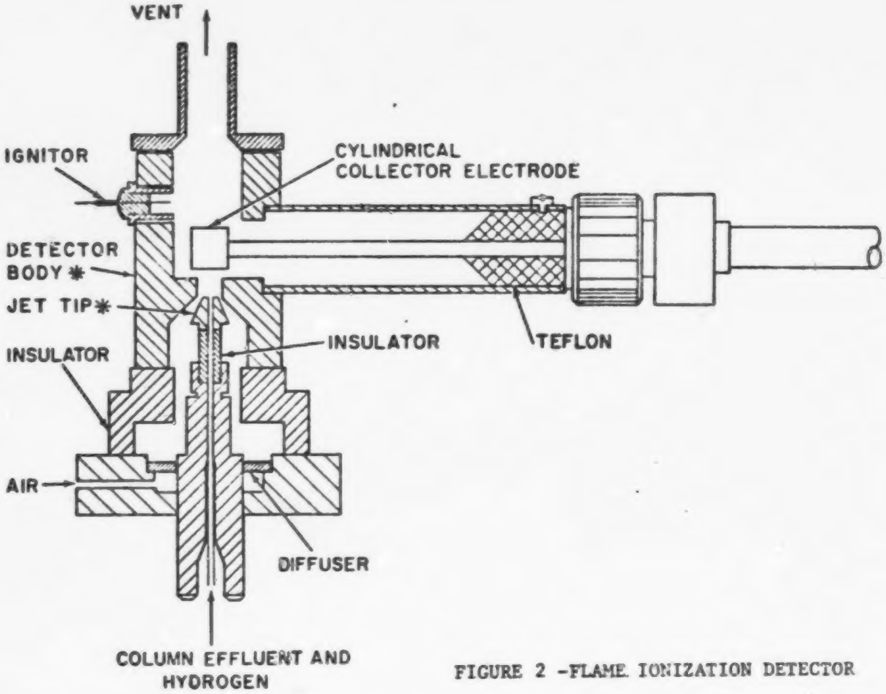

VENT
IGNITOR
CYLINDRICAL
COLLECTOR ELECTRODE
DETECTOR
BODY ✱
JET TIP✱
INSULATOR
INSULATOR
TEFLON
AIR
DIFFUSER
COLUMN EFFLUENT AND
HYDROGEN

FIGURE 2 -FLAME IONIZATION DETECTOR

TABLE 1 -COMPOUNDS GIVING LITTLE OR NO RESPONSE IN THE FLAME IONIZATION DETECTOR

He	No
O_2	N_2O
N_2	N_2O
CS_2	CO
H_2S	CO_2
SO_2	H_2O

Figure 3 shows the relation between FID response (in coulombs per mole) and hydrogen flow rate (ml/min). The carrier gas flow rate was 30 ml/min for the curves shown. Figure 3 shows that FID response is a sensitive function of hydrogen flow rate, and that individual detectors should be calibrated to determine the hydrogen flow rate that gives maximum response.

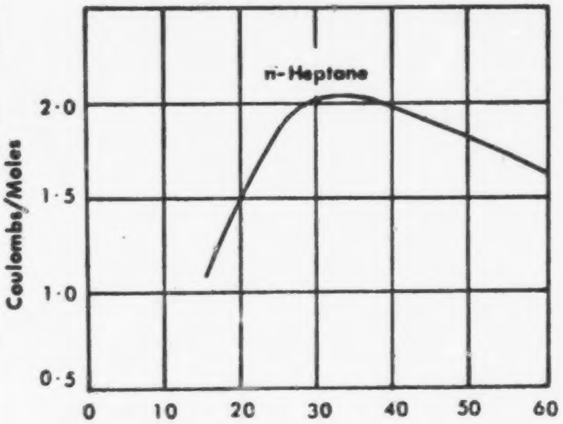


FIGURE 3 -F.I.D. SENSITIVITY VS. H_2 FLOW

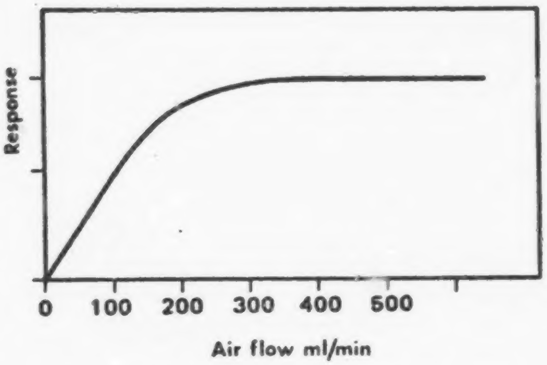


FIGURE 4 -F.I.D. SENSITIVITY vs AIR FLOW

One should increase both the hydrogen and air flow until a plateau of response is reached. Usually this is about 30 ml H_2/min and 350 ml air/min.

D Linear range

The FID has the widest linear range of any detector in common use. Linear range is between 10^6 and 10^7. The combination of high sensitivity and wide linear range make the FID the choice in trace analysis.

III Electron capture detector

A Operating principles

The electron capture detector measures the loss of signal rather than a positively produced electrical current. As the nitrogen carrier gas flows through the detector, a tritium source ionizes the nitrogen molecules and slow electrons are formed. These slow electrons migrate to the anode under the fixed cell voltage. Collected, these slow electrons produce a steady current amplified by the electrometer. If a sample containing electron absorbing molecules is then introduced, this current will be reduced. The loss of current is a measure of the amount and electron affinity of the compound.

B Detector response

The electron capture detector is extremely sensitive to certain molecules, such as alkyl halides, conjugated carbonyls, nitriles, nitrates, and organometals, but is virtually insensitive to hydrocarbons, alcohols, ketones, etc. Selective sensitivity to halides makes this detector especially valuable for the analysis of pesticides. Recent studies have shown that certain pesticides can be detected down to the sub-picogram quantity (10^{-13} gms).

C Radioactive source

Both H^3 (tritium) and Ni^{63} have been employed in the electron capture detector. The tritium E.C.D. was the first and remains the most frequently used detector. In general, it suffers from a lack of ruggedness because of a buildup of contamination on the surface of the radioactive foil. The Ni^{63} source can be heated to 350°C, thus minimizing this contamination effect. In addition, the column operating temperature can be much higher. The Ni^{63} is more expensive

FLAME IONIZATION DETECTOR SUMMARY

Minimum Det. Quant.	1 to 10 x 10^{-12} gm/sec.
Response	Sensitive to organic compounds. not to fixed gases or water
Linearity	10^6 to 10^7
Stability	Excellent (relatively insensitive to temperature and flow changes).
Temperature Limit	400°C
Carrier Gas	Nitrogen or helium

Summary: Inexpensive, simple, rugged, and can withstand extreme temperature change. Insensitive to fixed gases. Insensitive to water making it especially useful for analyzing dilute aqueous solutions.

however. The tritium foil is limited to 220°C because of radioactive leakage.

TABLE 2 -ISOTOPE CHARACTERISTICS

	H^3	Ni^{63}
Half life:	12.5 years	125 years
Particle Energy:	18 kev, B	67 kev, B
Specific Activity:	9,800 curies/gram	5 curies/gram
Physical Properties:	gas	solid
Foil Temp. Limitation:	220°C	350°C
Cost Per Curie:	$0.24	$17.5

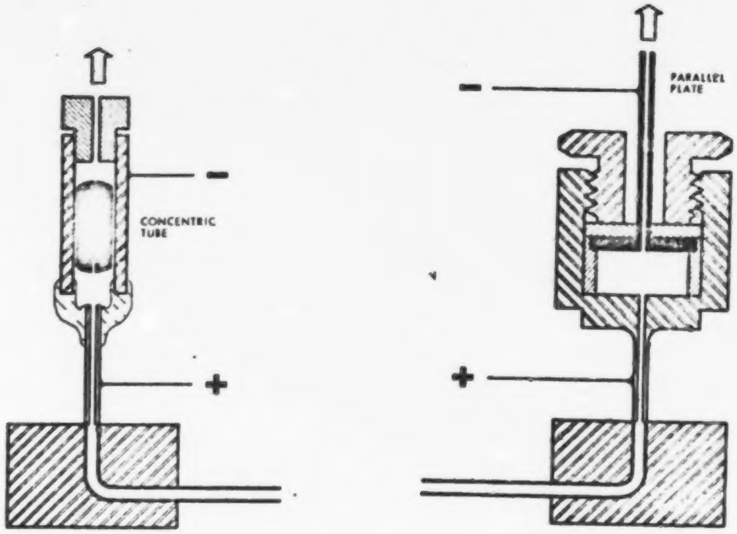


FIGURE 5 -ELECTRON CAPTURE DETECTOR

ELECTRON CAPTURE DETECTOR SUMMARY

Minimum Det. Quantity	10^{-13}g
Response	Variable sensitivity
Linearity	500
Stability	Fair
Temperature Limit	225°C (tritium) 350 for Ni^{63}
Carrier Gas	N_2 or Argon + 10% CH_4 (with pulsed voltage)

Summary - Detector is easily contaminated. Sensitive to water; carrier gas must be dry. Can be operated in pulsed or D.C. mode; non-destructive, most cells use a radioactive source requiring an AEC license.

IV Helium detector (2)

The Helium detector was developed for the trace analysis of permanent gases. It employs the cross-section detector geometry, 400 v electrode potential, and helium as carrier gas.

The combination of the tritium beta radiation and high field gradient (4000 volts per cm) raise the He to a metastable state with an ionization potential of 19.8 eV. All compounds having a lower ionization potential will be ionized giving rise to a positive signal.

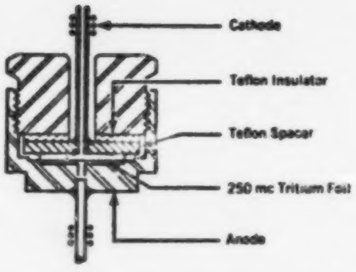


FIGURE 6 -HELIUM DETECTOR

HELIUM DETECTOR SUMMARY

Minimum Det. Quantity	10^{-12}g
Response	Sensitive to all compounds. Useful primarily for fixed gases.
Linearity	10^4
Stability	Poor (temperature and flow sensitive)
Temperature Limit	225°C
Carrier Gas	Ultra pure and dry Helium

Summary - Extreme sensitivity requires extremely clean system free from water vapor, column bleed, and back diffusion of air; expensive; can be used only with Porapak and active solid columns.

V Phosphorus detector

The recent de-emphasis in the use of chlorinated hydrocarbon pesticides has resulted in increased use of organo-phosphate pesticides. This led to work on selective phosphorus detectors (3,4). The phosphorus detector consists of a normal flame detector plus the addition of a small alkali salt pellet placed on the quartz burner jet.

With precise control of the hydrogen and air flow rates, this detector can be made to be very sensitive to phosphorus containing compounds and completely insensitive to other organic materials.

This modification resulted in a selective detector whose phosphorous response relative to the flame ionization detector is approximately 3000:1.

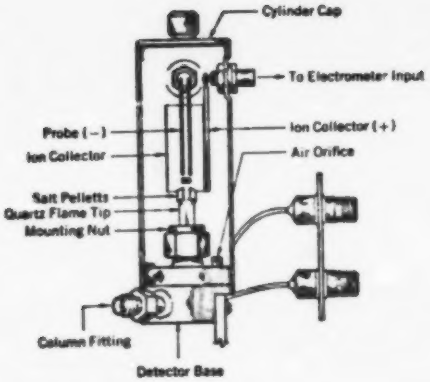

Cylinder Cap
To Electrometer Input
Probe (–)
Ion Collector
Ion Collector (+)
Air Orifice
Salt Pelletts
Quartz Flame Tip
Mounting Nut
Column Fitting
Detector Base

FIGURE 7 -PHOSPHORUS DETECTOR

PHOSPHORUS DETECTOR SUMMARY

Minimum Det. Quantity	10^{-11}g (parathi
Response	Sensitive to phosp compounds.
Linearity	10^{4}
Stability	Fair
Temperature Limit	300°C
Carrier Gas	Nitrogen or Helium

Summary - Moderate cost (requires flow controller for H_2 and air), selective and high sensitivity; destructive, fair stability, good linearity.

This outline was taken from the manual, Gas Chromatographic Analysis of Air Pollutants; Office of Air and Water Programs, Air Pollution Training Institute, Research Triangle Park, North Carolina 27711.

Descriptors: Analytical Techniques, Chemical Analysis, Chromatography, Gas Chromatography, Instumentation, Water Analysis

THERMAL CONDUCTIVITY

I. Theory of operation

The thermal conductivity detector is based on the principle that a hot body will lose heat at a rate which depends upon the composition of the surrounding gas. Thus, the rate of heat loss can be used as a measure of the gas composition.

Figure 1 shows a typical TC cell consisting of a spiral metal filament supported inside a cavity.

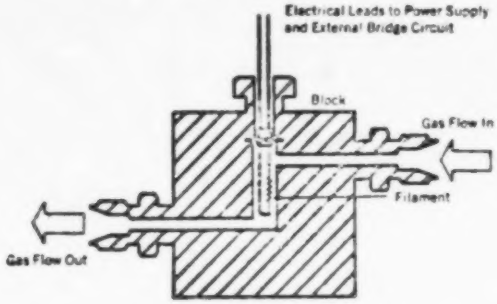

Electrical Leads to Power Supply and External Bridge Circuit
Block
Gas Flow In
Filament
Gas Flow Out

Figure 1 - Schematic of T.C. Cell

The heated filament can lose heat by the following processes:

1. Thermal conduction to the gas stream
2. Convection (free and forced)
3. Radiation
4. Conduction through the metal contacts

The major heat loss processes, however, are gaseous thermal conduction and forced convection. These two processes account for 75% or more of the total filament heat loss. Use of a carrier gas such as helium or hydrogen, will cause heat loss by gaseous thermal conduction to predominate. It is assumed in the following discussion that thermal conduction by the carrier gas is the only mode of heat transfer.

Heat is transferred by conduction when gas molecules strike the heated filament. The greater the number of molecular collisions with the filament per unit time, the greater the rate of heat loss. Differences in thermal conductivity of gases are based on the mobility or speed at which the gas molecules diffuses the smaller the molecule the higher its speed and the higher its thermal conductivity. Thus, hydrogen and helium which are the smallest molecules have the highest thermal conductivity. Table 1 gives the thermal conductivities (in c.g.s. units and at 0°C) of several common gases and shows how thermal conductivity decreases with increasing molecular weight.

II. TC sensing elements

A TC Cell consists of a spiral filament wire, (see figure 2) supported inside a cavity within a metal block. The filament is made of a material whose electrical resistance varies greatly with temperature, i.e., it has a high temperature coefficient of resistance. A constant current is passed through the filament causing its temperature to rise. In a typical TC cell, with helium carrier gas and a filament current of 175 milliamperes, the filament may reach a temperature of 100°C above the block temperature. With pure carrier gas flowing, the heat loss is constant and thus the filament temperature is also constant.

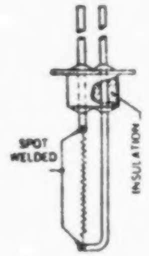


Figure 2 - GOW -MAC Filament

When a sample peak emerges, the filament temperature changes, causing a corresponding change in electrical resistance. It is this resistance change which is measured by a Wheatstone bridge circuit. Filament metals are chosen on the basis of high temperature coefficient of resistance, and resistance to chemical corrosion. Common filament metals are

TABLE 1 - THERMAL CONDUCTIVITY

Compound	Thermal Conductivity $\lambda \times 10^5$	Molecular weight
Hydrogen	41.6	2
Helium	34.8	4
Methane	7.2	16
Nitrogen	5.8	28
Pentane	3.1	72
Hexane	3.0	86

platinum, tungsten, and tungsten alloys.

III. Electrical circuit

The change in filament resistance must be measured and converted to an electrical signal. Figure 3 shows a simple Wheatstone bridge circuit. When all four filaments S_1, S_2, R_1, and R_2 are at the same temperature, the bridge is balanced and there is no signal. However, if the resistance of filaments S_1 and S_2 changes due to a change in gas composition, an unbalance occurs and an output signal is generated. Most detector blocks contain a pair of matched filaments in the sample flow channel, and a similar pair of matched filaments in the reference flow channel. The reference and sample flow channels are drilled into a single metal block having a high heat capacity. This provides temperature stabilization.

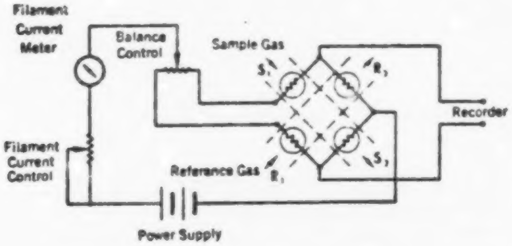


Figure 3 - T.C. Wheatstone Bridge Circuit

IV. Factors affecting sensitivity

Equation (1) gives the sensitivity of a TC cell in terms of the cell parameters:

$$S = K \cdot I^2 \cdot R \quad \frac{(\lambda c - \lambda s)}{\lambda c} \quad (T_f - T_b) \tag{1}$$

where:

S = sensitivity

K = cell constant dependent on geometry

I = filament current

R = filament resistance

λ_c = thermal conductivity of carrier gas

λ_s = thermal conductivity of sample gas

T_f = temperature of filament

T_b = temperature of detector block

A. Current

The I^2 term shows that increasing the filament current will increase the output signal significantly. In addition, an increase in filament current corresponds to an increase in filament temperature, and an increase in filament resistance R. The net result is a four-to eight fold increase in sensitivity for a two-fold increase in filament current. Of course, increasing current too high produces baseline instability and may burn out the filament.

B. Carrier gas

The term $\frac{(\lambda c - \lambda s)}{\lambda c}$ increases as λ_c gets larger. Therefore, choose a carrier gas having the highest possible thermal conductivity. Hydrogen or helium provide the highest sensitivity for organic compounds.

C. Temperature

Increasing the filament temperature T_f (by increasing filament current I) will increase detector sensitivity. However, the block temperature should be kept as low as possible, so as to maximize the difference $(T_f - T_b)$. The block temperature should be high enough to avoid condensation of the sample within the detector.

D. Summary

To increase the sensitivity of a TC detector, one should increase the filament current, decrease the block temperature and choose a carrier gas having high thermal conductivity.

V. Operating Suggestions for TC detectors

A. Always be sure that carrier gas is flowing through the detector before turning on the filament current. It is very easy to burn out the filaments unless a gas stream is present to dissipate heat.

B. Turn off the filament current before changing columns, putting in new injector septum, or otherwise opening the flow system to the atmosphere. Small volumes of air leaking into the system can oxidize and ruin the heated filaments.

C. Excessive noise, baseline drift, or inability to balance the TC bridge may be caused by corrosion of the filaments. If filaments are badly corroded they must be replaced. However, if baseline starts to drift (after being straight), immediately turn off filament current and check the system for leaks.

D. Excessive noise and baseline drift may also be caused by high boiling components which have condensed on the filaments. Cool the detector block to room temperature, disconnect the column, and place an injector septum and nut on the detector inlet. Inject sufficient solvent (benzene or xylene) to fill the flow channels, let stand overnight. Hot xylene is a good solvent for silicone polymers. Clean and dry thoroughly before using.

E. Samples such as HCl, chlorine, fluorine, alkyl halides, organo-fluorides, and other reactive compounds will readily damage standard TC filaments. If working with such compounds, use a nickel detector block equipped with Teflon-coated filaments. There is a two to three fold loss in sensitivity.

F. TC detectors are flow sensitive. The carrier flow rate should be held constant by means of a two-stage regulator. Temperature programming with a TC detector requires a differential flow controller because of the expansion of the carrier gas with increasing temperature.

Descriptors: Analytical Techniques, Chemical Analysis, Chromatography, Gas Chromatography, Instrumentation, Water Analysis

This outline was taken from the manual, Gas Chromatographic Analysis of Air Pollutants; Office of Air and Water Programs, Air Pollution Training Institute, Research Triangle Park, North Carolina 27711

SPECIAL DETECTORS

I. Flame photometric detector

The flame photometric detector (FPD)[1] (patent pending), developed by Brody and Chaney is shown in Figure 1, and Figure 2.

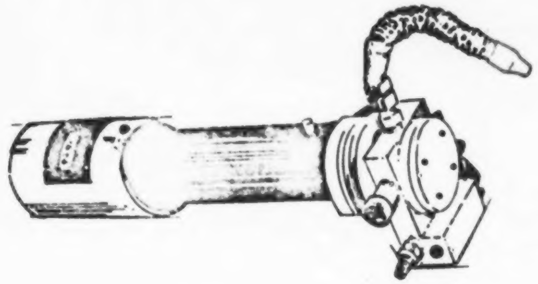


FIGURE 1. FLAME PHOTOMETRIC DETECTOR

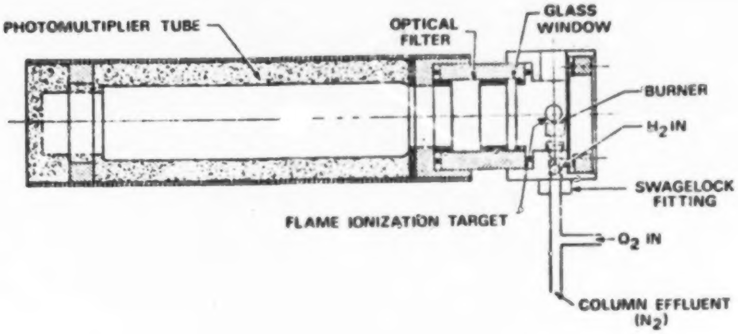


FIGURE 2. FLAME PHOTOMETRIC DETECTOR SCHEMATIC

In this detector, the column effluent is mixed with an air/oxygen mixture and combusted in a flame surrounded by an envelope of hydrogen, the flame being seated within the confines of a high-walled cup. Organic species in the sample are fragmented and excited to higher energy states in the reducing atmosphere of the flame. The plasma above the cup is viewed by a conventional photomultiplier tube through a narrow bandpass filter of the appropriate wavelength according to the element of interest.

A collector electrode above the flame provides a simultaneous flame ionization output. A single sample injection thus gives a standard flame ionization trace as well as that for sulfur or phosphorus components. Of even greater versatility is the dual filter, dual photomultiplier version of the flame photometric detector, which permits simultaneous readout of sulfur, phosphorus, and flame ionization response.

This system insures maximum sensitivity toward sulfur and phosphorus emissions without significant interference from hydrocarbon species. The interference filters are chosen to have maximum transmittance at either 526 mμ, corresponding to the wavelength of phosphorus HOP species in the hydrogen-rich flame, or 394 mμ, which is the prime wavelength of sulfur S_2 emissions. The selection of these wavelengths allows the detection of P or S in organic compounds with a minimum of intereference, and the inherent sensitivity of flame spectroscopic techniques provides high sensitivity. The present detection limit of the FPD is 10 pg of phosphorus and 40 pg of sulfur.

Some typical applications of the flame photometric detector are given in Figs. 3-5. Figure 3 is a chromatogram of commercial premium gasoline that contains tricresyl phosphate. The cluster of peaks on the FPD channel represents an amount of phosphorus compounds too small to be detected by the flame ionization detector (FID). Figure 4 shows the analysis of three aliphatic sulfides at the 50-ppm level in

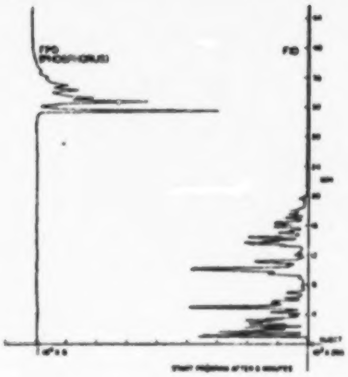


FIGURE 3. FPD analysis of tricresyl phosphate in commercial gasoline. Sample: 1 1. Column: 1/4-inches X 6-ft glass, OV-1 on chromosorb W-HP. Column temperature: 50°-250°C at 5°C/min.

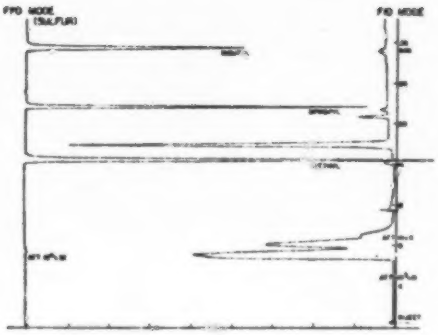


FIGURE 4. FPD analysis of aliphatic sulfides in hexane-diethyl sulfide, di-n-propyl sulfide, and di-n-butyl sulfide. Sample: 1 1 (50 ng 1 1). Column: 1/4-in. X 10-ft. glass, carbowax on chromosorb-HP. Column temperature: 50°-200°C at 10°C/min. Carrier gas: nitrogen, 45 ml/min.

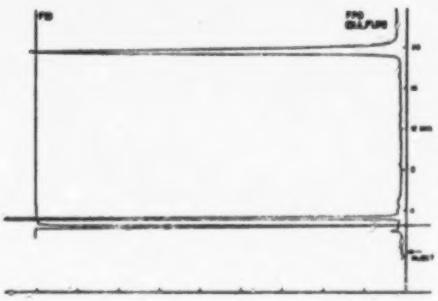


FIGURE 5. FPD analysis of sulfolane in tetradecane. Sample: 0.5 1 (50 ng/ 1). Column: 1/4-in. X 10-ft carbowax on chromosorb-HP. Column temperature: 225°C. Carrier gas: nitrogen, 80 ml/min.

hexane. At this concentration, the FID is barely able to detect the sulfur compounds, whereas the FPD responds greatly. Figure 5 depicts the analysis of sulfolane (tetrahydrothiophene-1, 1-dioxide) in n-tetradecane; the sulfur response shown represents 25 ng of sulfolane. The FPD peak at the start of the chromatogram is caused by the large amount of hydrocarbon producing an interference.

In addition to providing a means of detecting compounds that do not contain phosphorus or sulfur, the FID output is used in conjunction with the FPD response to determine if unknown compounds contain sulfur or phosphorus. The equation is

$$E = \frac{(s/n)\ FID}{(s/n)\ FPD}$$

E is the element selectivity and s/n represents the signal-to-noise ratio of peaks in question as recorded on the dual system. The equivalent noise level in each case is measured by altering the attenuation to give significant noise (1-3%) and including the actual attenuator setting for observed peak height in the calculation.

Consider for example the dibutyl sulfide peak in Figure 4. On the FID channel a peak of 1.5 chart divisions was observed with attenuation of 10 X 4, and a 1% noise level could be observed with attenuation of 1 X 4. On the FPD channel a peak of 53

Table 1
Selectivity of the flame photometric detector

Compound Type	Relative amount required to give equal response with S
Hydrocarbons, aliphatic	10,000
Hydrocarbons, aromatic	10,000
Oxygen compounds	5,000
Nitrogen compounds	5,000
Chlorine compounds	40,000

chart divisions was observed with attenuation $10^4 \times 32$, and a 2% noise level could be observed with attenuation of $10^3 \times 4$. Substitution of these values into the equation gives

$$E = \frac{\dfrac{1.5 \times 10 \times 4}{1 \times 1 \times 4}}{\dfrac{53 \times 10^4 \times 32}{2 \times 10^3 \times 4}} = \frac{15}{2120} = 0.007$$

In general, E will be less than 5 for sulfur or phosphorus compounds and at least 1000 for a hydrocarbon. In this way, positive confirmation regarding the presence of a sulfur or phosphorus compound is made. This typical element selectivity of the flame photometric detector toward sulfur and phosphorus over other common compounds is listed in Table 1.

II Coulsen Electrolytic Conductivity Dectector[1].

The principle of its operation is demonstrated by the diagram of an electrolytic conductivity gas chromatograph shown in Figure 6.

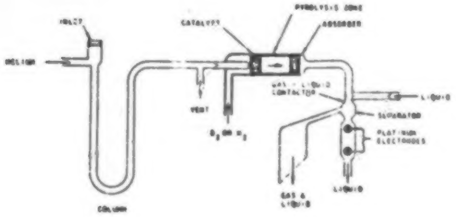


FIGURE 6. ELECTROLYTIC CONDUCTIVITY GAS CHROMATOGRAPH

The system consists of a pyrolyzer, a gas-liquid contactor, a gas-liquid separator, and a simple pair of platinum electrodes in a d.c. bridge circuit. The pyrolyzer converts the organically bound halogen, sulfur, or nitrogen to oxidized or reduced substances that form electrolytes when dissolved in water. These electrolytes are detected by the change they cause in the conductivity of water in the detector cell. Conductivity is measured between the two platinum electrodes of the cell by means of a simple d.c. bridge and recorded continuously on a 1 mv strip-chart recorder.

A. Chlorinated hydrocarbon pesticides like DDT may be burned with moist oxygen in the pyrolysis tube to form hydrogen halides giving strong electrolytes when dissolved in water. As little as 1 nanogram of hydrogen chloride from chlorinated compounds can readily be detected in the presence of much larger quantities of non-halogen-containing organic compounds. A typical gas chromatogram for chlorinated hydrocarbons with the Coulson electrolytic conductivity detector is shown in Figure 7. In this case, oxidative pyrolysis with moist oxygen and a platinum catalyst was used. Identical results were obtained for this sample with reductive pyrolysis. In both oxidative and reductive pyrolysis the furnace temperature was 850°C.

Sulfur compounds also give strong response in the electrolytic conductivity detector in the oxidative mode but not in the reductive mode. Thus, the most specific results for halogenated compounds are observed in the reductive mode with a platinum catalyst. Under these conditions there is little or no response from the elements N,S, P, C,O and H in organic molecules.

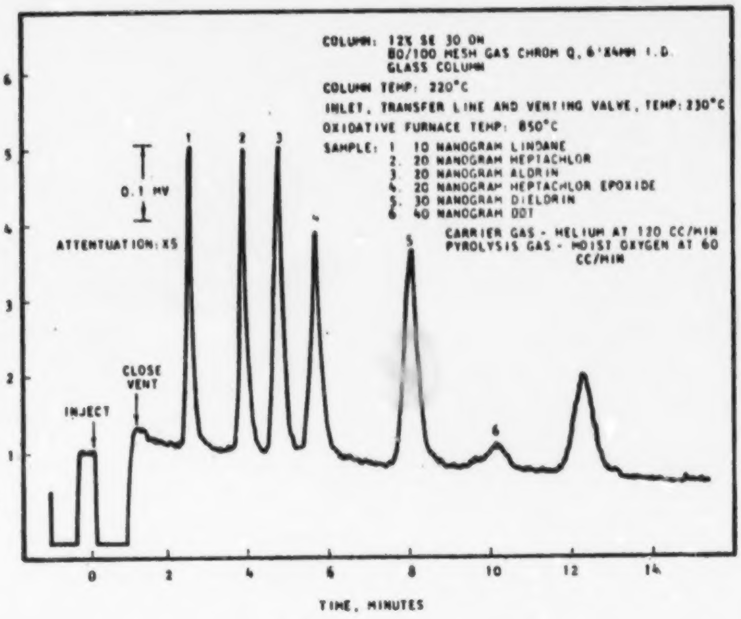


FIGURE 7. ELECTROLYTIC CONDUCTIVITY
GAS CHROMATOGRAM

The excellent specificity of the electrolytic conductivity detector for halogens in the reductive mode is demonstrated in Figure 8. This sample was a poorly cleaned-up extract of a rodent that had been exposed to chlorinated hydrocarbon pesticides. It was not sufficiently cleaned-up to give a satisfactory electron capture gas chromatogram as indicated in curve A in Figure 8. It was too dirty to give satisfactory curves with oxidative pyrolysis conditions with either microcoulometry or electrolytic conductivity. Curve B with reductive pyrolysis conditions in the electrolytic conductivity system shows well defined peaks for the chlorinated pesticide residues including 0.3 ppm of DDE, a trace of DDT, and an unidentified component of earlier elution.

B. One of the more promising uses of electrolytic conductivity gas chromatography is lective and sensitive analysis for organic nitrogen compounds. Figure 9 is a curve for a 20-microliter sample of acetone containing 25 nanograms of azobenzene (3.8 nanograms of nitrogen) and 100 nanograms of parathion (5 nanograms of nitrogen). This sample also contained 50 nanograms of lindane, and 100 nanograms each of heptachlor, aldrin, and heptachlor epoxide. In this mode of operation, which used a nickel wire catalyst and strontium hydroxide coated on Fiberfax as acid absorber, nitrogen was the only element detected.

The electrolytic conductivity detector is presently being used to study chlorinated hydrocarbon and organic nitrogen-containing pesticide residues, flavors and fragrances, and amino acids. It will find wide use in analyzing pharmaceuticals, chemicals, petroleum and petrochemicals, and biological materials.

III. Microcoulometric Detector

A microcoulometer is used for the quantitative measurement of the chloride content of chlorinated hydrocarbons

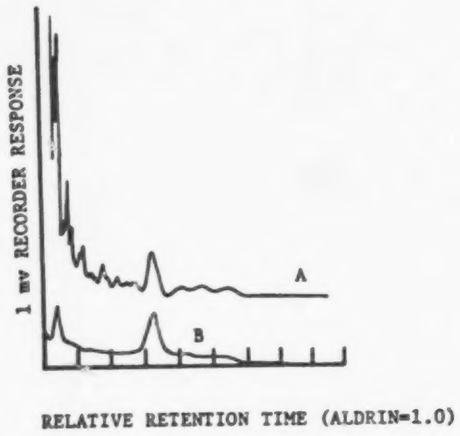


FIGURE 8. ELECTRON CAPTURE AND ELECTROLYTIC CONDUCTIVITY CHROMATOGRAMS

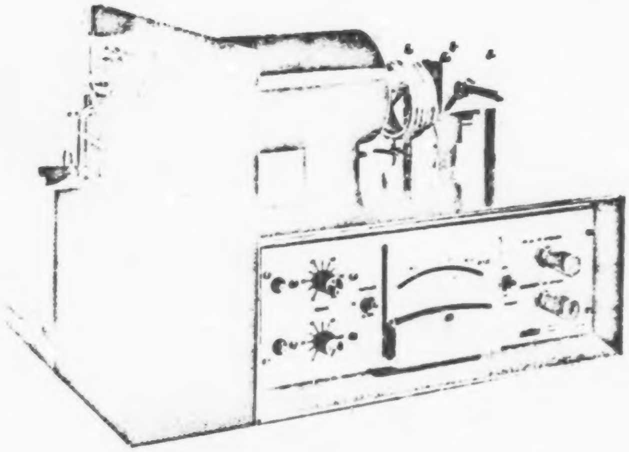


FIGURE 9. COULSON ELECTROLYTIC CONDUCTIVITY DETECTOR

eluted from the gas chromatography. A schematic diagram of the microcoulometric gas chromatograph is given in Figure 10.

Gases eluted from gas chromatographic separation are mixed with oxygen in the combustion furnace and burned to CO_2, H_2O, HCl and inorganics.

These products are bubbled into a titration cell in which there is a continuous titration of the chloride ions with Ag ions. The Ag ions are electrically generated in the titration cell so that the concentration remains constant throughout the titration.

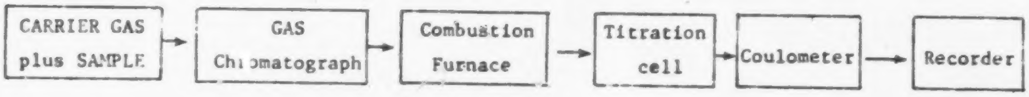


FIGURE 10. MICROCOULOMETRIC GAS CHROMATOGRAPH

A graphic representation of the current required to maintain the concentration of Ag ions vs. time is recorded.

The total quantity of electricity (coulombs) is calculated from the area under the peak and this can be directly related to the concentration of halogenated hydrocarbon using Faraday's constant.

Response of coulometer to halide ions is linear and quantitative throughout the range of 0.15 to 10.4 micrograms.

Less than quantitative recoveries are obtained for some pesticides during extraction and manipulation of samples.

SENSITIVITY AND SPECIFICITY

Sensitivity of new model microcoulometer is 1 nanogram chloride ion for 5% full-scale deflection.

Sensitivity for sulfur is approximately similar.

Sensitivity for pesticide depends on chlorine or sulfur content of molecule.

Coulometer will respond only to halide (except fluoride) and sulfur. There is no evidence of response to other possible products of combustion, with the exception of sulfite ion.

Descriptors: Analytical Techniques, Chemical Analysis, Chromatography, Gas Chromatography, Instrumentation, Water Analysis

This outline was taken from the manual, Gas Chromatographic Analysis of Air Pollutants; Office of Air and Water Programs, Air Pollution Training Institute, Research Triangle Park, North Carolina 27711

CHARACTERISTICS OF THE FLAME IONIZATION DETECTOR

When gases burn in a flame and become sufficiently hot a small percentage of the molecules ionize and give rise to an electrical conductivity within the flame. When an organic molecule enters this flame it causes this conductivity to increase. This increased conductivity is measured and recorded. Thus, we have the basis for a very sensitive method for the detection of organic material. The credit for this observation is given to Harley, Nel, and Pretorius and to McWilliam and Dewar(1, 2, 3)

The exact mechanism within the flame which is responsible for the conductivity has been the subject of considerable smoke and very little light and for this reason will not be discussed here. Interested parties will want to consider references 4, 5, 6, 7, and 8 for further information on this point. It should be mentioned however that the efficiency of the flame ionization detector (FID) is very small having an upper limit of approximately 0.01% and ranging down to 0.0001% for most common commercial detectors.

The electrical characteristics of the FID should be mentioned since they give us a clue as to its best mode of operation. In normal practice one usually puts 200 to 300 volts across the electrodes to collect the ions. The resistance of a hydrogen flame is of the order of 10^{-14}ohms while the background current is approximately 10^{-12} amperes and the signal current may run up to 10^{-7}amperes. The measurement of such small currents demands that we use a high impedance amplifier (electrometer) and some care in the design of the FID itself along with the associated circuity.

SENSITIVITY AND LINEARITY

It should be kept in mind that the FID is a mass flow detector rather than a concentration detector. This means that the response of the flame is dependent on the number of grams of carbon which enter the flame per second rather than the concentration of carbon in the carrier gas entering the flame. The limit of detection for this device is expressed in such terms as 1 in 10^{-9}molar, one part per billion, 2×10^{-13} gram atoms of carbon, or 5×10^{-12} grams of carbon per second. The linearity of the flame commences at detectability and runs to at least 0.5% while the dynamic range covers four to six orders of magnitude.

CALIBRATION

This device demands careful calibration and operation for good quantitative results as shown by the fact that the molar response for 2, 2, 4-trimethylpentane is 30% greater than that for ethyl benzene and 15% greater than that for n-octane (all C_8 molecules).

One usually calibrates a FID by determining response factors for the compounds of interest. This response depends on geometry and operating conditions of the FID and is usually expressed in coulombs per gram of carbon or in coulombs per gram atom of carbon. In the ultimate analysis what we wish to know is the absolute or relative amount of material leaving the chromatographic column. This amount is the integral of the detector current with respect to time providing the detector signal and mass flow rate are linear over the range in question.

Figure 1 and Equation 1 illustrate what we are measuring.

$$I = r \times g \times t^{-1} \qquad (1)$$

where:

I = amperes

r = coulombs/gram

g = grams of carbon

t = time in seconds

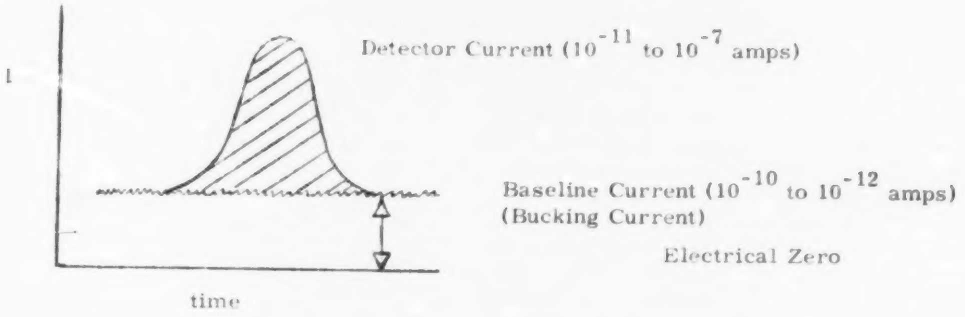


Figure 1

OPERATING PARAMETERS OF THE FID

In general the electrode geometry and polarizing voltage for the detector are determined by the manufacture and you have very little control over them. This is, however, an important point and should not be overlooked in the purchase of new equipment.

GASES USED

When using a FID and working in the low parts per billion region certain problems become acute. Foremost among these is the need for "clean" gases (hydrogen, air, nitrogen or helium). Using proper techniques(9) it is possible to get sensitivity to the low parts per billion range.

For hydrogen:	Use a palladium-silver diffusion purifier or a liquid nitrogen cold trap changed with Davison 912 silica gel.
For air:	Air taken through a combustion furnace at 825° C and then over a drying agent such as Drierite.
For helium:	Use a liquid nitrogen trap; still some residual methane gets through.

TYPICAL GAS FLOW RATES FOR FID

The values listed below are "average" values. For maximum sensitivity one must experimentally determine what the best flow rates are for each individual detector.

Scavenger gas:	Either air or oxygen may be used at a flow rate of between 200 and 500 cc/minute depending on the geometry and flow patterns within the detector.
Fuel gas:	Hydrogen is normally used at a rate given by: $H_2 = \frac{1}{3}$ (carrier gas + 10) cc/minute
Carrier gas:	Usually helium or nitrogen is used at a rate given by: Carrier gas = 3(H_2flow - 10) cc/minute Many times this flow rate is determined by chromatographic conditions and it may be necessary to add "make up" gas between the end of the column and the flame detector.

OPTIMIZING FID PERFORMANCE

1) Dependence of ion current on polarizing voltage
2) Dependence of ion current on mass flow rate of sample
3) Dependence of ion current on scavenger gas rate
4) Dependence of ion current on fuel gas rate
5) Dependence of normalization factors on fuel gas rate

CONCLUSION

A great deal of information is available today on the design and operation of flame detectors. In order to do good quantitative work the operator of such a detector must take the time to understand its operation and limitations. Used with care this detector will give very good results - however, if used in an indiscriminate manner it will lead to results which may contain large errors. The type of work you do will depend on your understanding of this detector.(10)

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This outline was prepared by W.E. Baitinger, Department of Chemistry, Purdue University, Lafayette, Indiana.

Descriptors: Chemical Analysis, Chromatography, Gas Chromatography, Instrumentation, Water Analysis

PREPARING GAS CHROMATOGRAPHY COLUMNS

I INTRODUCTION

A The object of this laboratory demonstration is to familiarize the student with columns and packing materials, and procedures used in preparation of columns for pesticide residue analyses using the electron affinity detector.

B Materials

1 Columns

a Stainless steel

b Aluminum

c Quartz

d Glass

2 Solid support

a Diatomaceous earth

1) Chromosorb, regular
2) Chromosorb P

a) Pink
b) Calcinated (Powdered by heating at high temperatures)

3) Chromosorb W

a) White
b) Flux-calcinated (Heated at high temperatures with about 3% sodium carbonate)

4) Anakrom

a) White
b) Flux-calcinated
c) Silanized

5) Acid washed chromosorbs

a) Washed with concentrated hydrochloric acid
b) Removes traces of aluminum, iron, magnesium, and calcium, which are present in diatomaceous earth

b Firebrick

1) Firebrick, regular
2) Firebrick, acid washed

c Polytetrafluoroethylene (Teflon)

1) Fluoropak
2) Teflon 6
3) Neutraport
4) Haloport

3 Stationary phase (Immobile phase)

a Silicone Dow 11

b Silicone SE-30

c Epikote

d Apiezon L

II PROCEDURES USED TO IMPROVE THE SOLID SUPPORT

A Separation of Materials into Narrow Range Particle Sizes

B Removal of Foreign Materials

1 Acid washing

2 Alkali washing

C Deactivation - Covering of Active Absorption Sites

1 High temperature

2 Sodium carbonate

3 Silicon dioxide

4 Hexamethyldisilazene coating

5 Epon coating

III COLUMN PREPARATION PROCEDURE

A Column

1 Select appropriate material.

2 Cut to desired length.

3 Weigh empty column and record weight.

4 Calculate the volume of the column in cubic centimeters (Quoted column diameter is outside diameter).

B Preparation of Packing Materials

1 Select the solid support.

2 Weigh out this material slightly in excess of that required to pack the column.

3 Select the desired stationary phase material.

4 Calculate the desired amount of stationary phase to use with the previously weighed solid support.

5 Dissolve the stationary phase material in an appropriate volatile solvent, such as ethyl acetate, in sufficient quantity to completely cover the solid support, leaving an excess of liquid phase.

6 Thoroughly mix the solid support and solvent containing the stationary phase.

7 Completely remove the solvent by means of a steam bath or rotating evaporator, with constant mixing.

8 Place the prepared packing material in a drying oven for several hours or overnight. A good general rule is to dry at about 90° C, temperature should not be allowed to reach 100° C.

C Packing the Column

1 Lightly pack one end of the column with a plug of glass wool.

2 By means of a tube, attach a small funnel to the opposite end of the column.

3 With a graduated cylinder, measure the volume of packing material which is equal to the volume of the column, tapping the cylinder to compact the material.

4 Weigh this volume of packing material and record the weight.

5 Slowly add this material to the column, running a small hand vibrator up and down the column or tapping the column on the floor to compact the packing.

6 Close the open end of the column with a plug of glass wool.

7 Attach the fittings to the column and install in the chromatograph column oven.

8 Check the carrier gas flow through the column at the operating gas pressure.

9 With the carrier gas flowing, condition the column by heating at a temperature about 50° C higher than operating temperature.

10 The period of time required for conditioning a column ranges from about 12 - 72 hours depending upon the packing materials used.

11 Appreciable up-scale drifting of the baseline is indicative of improper or insufficient conditioning of the column.

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Descriptors: Adsrobents, Chromatography, Gas Chromatography, Separation Techniques, Water Analysis

TROUBLE SHOOTING THE GAS CHROMATOGRAPH

I ROUTINE FOR DAILY OPERATION OF GAS CHROMATOGRAPH

A Turn on all electronics for warm-up.

B Check system for gas leaks and then adjust gas flow.

C Check recorder zero and note baseline at various sensitivities.

D Inject standards and compare with previous runs.

II PROBLEMS AND CAUSES (ELECTRON CAPTURE)

A Erratic baseline noise. (In excess of 4% of half of maximum sensitivity).

1 Column operating temperature too high.

2 Column substrate coating weight excessive (in excess of 7%).

3 Columns insufficiently conditioned.

4 Contaminated system.

5 Insufficient bakeout time with pure gas flowing.

6 Internal recorder signal connections improper.

7 Signal cable loose.

8 Loose ground.

9 Effluent end of Electron Capture Detector exposed to turbulent air.

10 Intermittent Electron Capture Detector heater.

11 Cracked Electron Capture Detector housing.

12 Effluent end of detector shorting to case.

B Slow Regular Noise

1 Insufficient pressure differential between carrier supply regulator and column head pressure at the maximum analytical column operating temperature.

2 Column temperature too high.

3 Condensation somewhere between end of column and effluent end of Electron Capture Detector.

4 Defective carrier flow regulator.

C Insufficient Sensitivity of Response

1 Using metal columns

2 Septum leaking

3 Bad leak

4 Cold spot

5 Diluent flow too high or too low.

6 Improper carrier gas.

7 Carrier gas contaminated with water.

8 Carrier gas contaminated with oxygen.

9 Operating Electron Capture Detector electrode potential in DC Mode incorrectly set.

10 Recorder incorrectly connected to instrument signal output terminals.

11 Depleted (weak) source element.

12 Pulse rate too fast, in pulse mode operation.

13 Pulse generator not operating, in pulse mode operation.

14 Cracked Electron Capture Detector housing.

15 Leaking Electron Capture Detector housing.

D Baseline Drift Upscale

1 Column temperature programming (thermal upscan).

E Baseline Drift Downscale

1 Contaminated sample system.

a The "proper solvent" is benzene, hexane, or similar non-adsorbing hydrocarbon solvent.

b Chlorinated or other electron absorbing solvents should be avoided except as a last resort in cases of extreme contamination.

c More generally the following solvents and cleaning method must be avoided:

1) Ethyl or Methyl Alcohol

2) Water

3) Organic Acids, Acetic Acid, etc.

4) Phenolic Solutions

5) Anything containing Labile, Acting Hydrogen.

6) Scrubbing the source by ultrasonic cleaning.

2 Column bleed.

F Excessive Tailing From Sample Solvent Peak

1 Premature vaporization of sample at in-side end of septum.

2 Solvent

3 Diluent gas flow rate insufficient.

4 Sample quantity excessive.

III PROBLEMS AND CAUSES (MICROCOULOMETRIC)

A Positive High Baseline

1 Titratable material entering cell.

B Negative Baseline

1 Silver from silver acetate electrode entering cell.

C Noisy Baseline

1 Stirring bar

D Baseline goes negative at irregular intervals and is slow returning

1 Excess silver in cell

E Erratic baseline

1 Temperature of cell varying.

F Peaks Overshoot

1 Under damping.

G Peaks overshoot but system acts over damped.

1 Dirty cell

H Peaks tail

1 Over damped

I Odd Peaks

  1 Contaminated system

J Peaks Break-up

  1 Contaminated system

K Low Recoveries

  1 Leaking system

  2 Improper combustion

This outline was prepared by J. I. Teasley, former Supervisory Chemist, Southeast Water Laboratory, EPA, Athens, GA.

Descriptors: Chemical Analysis, Chromatography, Instrumentation, Water Analysis

QUANTITATION IN GAS CHROMATOGRAPHY

I INTRODUCTION

One of the factors which has led to gas chromatography's rapid development in the field of instrument analysis is the quantitative precision with which samples can be analyzed. There are several equally important factors involved in quantitative analysis. These are: Accurate sample introduction, constant operating parameters, accuracy of peak area measurement, sensitivity factors of individual compounds, linearity of detector and columns which give well resolved peaks.

II CALIBRATION PROCEDURE

A Peak Height and Peak Area

Quantitative work is based on peak height or area. Peak height measurement is more rapid than peak area; however, plots of peak height vs. sample size are more non-linear than corresponding plots for peak area. This is because peak heights and widths are frequently dependent on sample size and sample feed volume, however; total area is not. Peak heights are generally used if samples are less than 10 μg for packed columns and 0.1 μg for capillary columns.

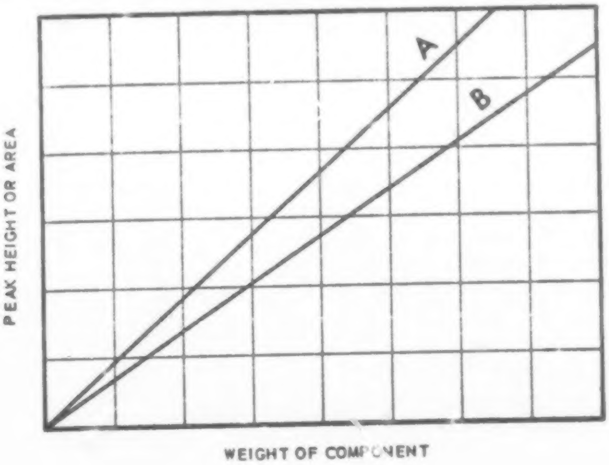
B Direct Calibration

Blends of components in question are prepared and chromatographed. The value of peak heights or areas are then plotted against the weight of sample injected. The unknown sample is then injected and its peak height or area is compared to that of the standard.

The main disadvantages of direct calibration are that the precise amount of sample injected must be known and that calibration is time consuming. Also, the sensitivity of the detector must remain constant from run to run and day to day in order to compare results with the calibration graph.

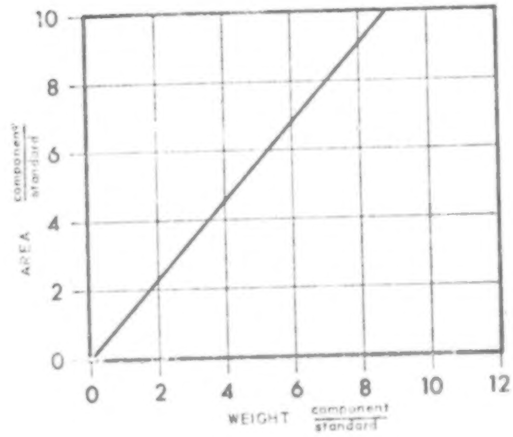
C Internal Standardization

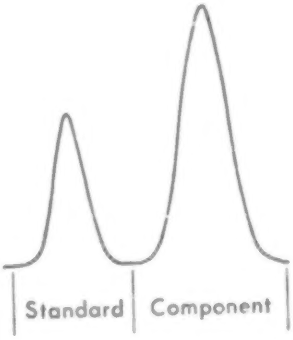
This is a common procedure used in biomedical analyses, such as alcohol in blood, and steroids in serum and urine. Known weight ratios of the component in question and a standard (marker) are prepared and chromatographed. The component/standard area ratios obtained are then plotted vs. the component/standard weight ratios.



To the unknown, add a known volume concentration of the standard. This mixture is then chromatographed and the component/standard area ratios are determined. By determining the corresponding component/standard weight ratio from the graph, the unknown amount of the component can be determined.

e.g.: To the unknown, add 5 ml of a solution containing the standard whose concentration is 100 μg/ml. Upon chromatographing the mixture, the area ratio was found to be 8; therefore, the weight ratio is 7 (see graph). Knowing standard concentration to be 100 μg/ml, then the component concentration is 7 × 100 μg/ml. Since we added 5 ml of standard solution, then the total amount of the unknown is 5 ml × 700 $\frac{\mu g}{ml}$ = 3500 μg or 3.5 mg.



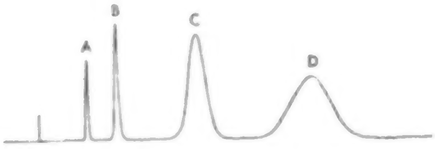


A particular advantage of using the internal standardization method is that the amount injected need not be accurately measured. Also, the detector response need not be known or remain constant since any change in sensitivity will not affect the area ratio.

The chief disadvantage of this method is the difficulty in finding a standard that does not interfere with a component in the sample. It is also time consuming.

D Internal Normalization

This is used when only approximate data are required. Assuming all the components have been eluted, the percent composition of a component within a mixture is its area divided by the sum of the areas.



$\%$ Composition $A = 100\left(\frac{A}{A+B+C+D}\right)$

This method also assumes that area percent equals weight percent. This may only be true when analyzing close boiling components of a homologous series. To obtain the weight percent, it is necessary to multiply each area by a correction factor.

III NORMALIZATION OF AREAS (CORRECTION FACTORS)

A Normalization for Thermal Conductivity Detectors

This procedure relies on the fact that each compound has a unique thermal conductivity cell response. For quantitative analysis, the thermal conductivity of the sample should be approximately a linear function of its composition in the range concerned. From the areas under each peak and the relative responses that are characteristic of each peak, it is possible to determine the quantity of each component in the sample.

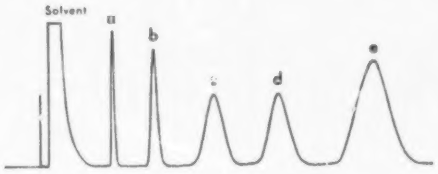
Sample calculation:

COMPOUND	AREA	RELATIVE T.C. RESPONSE PER MOLE (2)
Ethanol	5.0	72
Heptane	9.0	143
Benzene	4.0	100
Ethyl Acetate	7.0	111

			NORMALIZE		MOLE %
Ethanol	$\frac{5.0}{72}$	= 0.070	$\frac{0.070}{0.236}$	=	29.6
Heptane	$\frac{9.0}{143}$	= 0.063	$\frac{0.063}{0.236}$	=	26.7
Benzene	$\frac{4.0}{100}$	= 0.040	$\frac{0.040}{0.236}$	=	17.0
Ethyl Acetate	$\frac{7.0}{111}$	= 0.063	$\frac{0.063}{0.236}$	=	26.7
TOTAL		0.236			100.0%

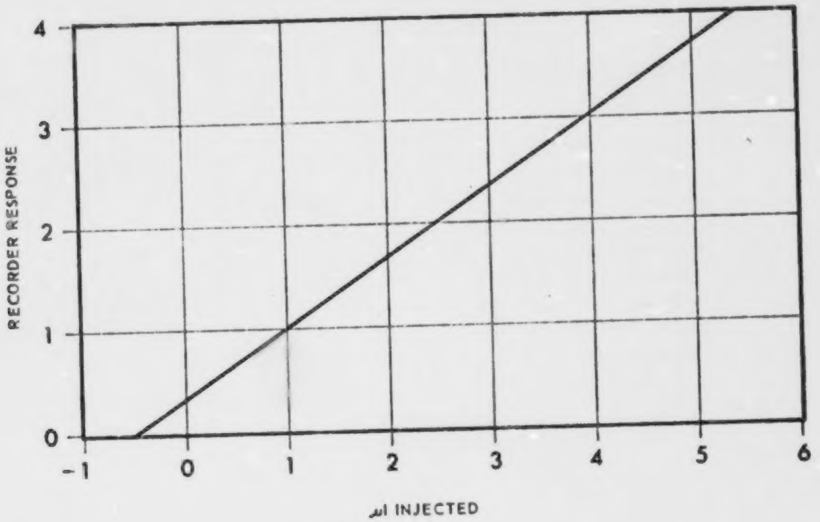
B Normalization for Mass Detectors

1 A standard solution of compounds a, b, c, d, and e in a solvent is prepared.



2 The weight percent is calculated and the known densities are used to convert percent.

3 Inject several samples and plot peak area (or height) vs. volume injected.



4 Note that intercept on "X" axis corresponds to an injection error of 0.5 µl. Therefore, actual amount injected = uncorrected + 0.5 µl. For 2.5 µl (corr.) injected, the density values are used to determine the actual weight W. injected.

5 The corresponding area of each peak is determined and the response in $cm^2/\mu g$ is determined.

6 Using compound "a" as standard (correction factor = 1), the corresponding correction factors are determined. See the Table on Page 8-6.

IV STATISTICAL TREATMENT OF DATA

A Accuracy and Precision Data (Reproducibility)(3, 4)

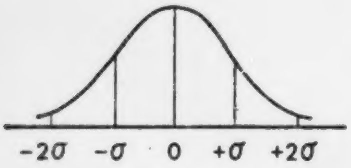
Accuracy is a measurement of the difference between the true value and the determined values. In those cases in which the true value is not known, it is necessary to express the exactness of a measurement in another way. This may be done by obtaining the average of a number of measurements and finding the difference (deviation) of each value from the average value. The magnitude of deviation is a measure of the precision of measurement.

It is seen that accuracy expresses the correctness of a measurement, whereas precision expresses the reproducibility of a measurement.

The accuracy and reproducibility achieved in gas chromatography depend on many things; among them, the correct choice of column, temperature, flow rate, sample size, detector, and injection system. Highest performance demands considerable understanding of the chromatographic process and of the effects of change in a large number of variables. However, even with ill-designed apparatus, analyses can usually be conducted with an accuracy better than ± 10 percent per component. When precautions are taken, an accuracy and reproducibility of about ± 1 percent per component is easily attainable.

B Errors

Errors fall into two classes. Determinate errors are those whose cause and magnitude can be determined. (Errors in method, apparatus, operator, etc.) Indeterminate errors are random errors which cannot be eliminated. The distribution of indeterminate errors follows the normal probability law as shown in the error curve. This curve shows that positive and negative deviations are equally probable and that small deviations occur much more frequently than large ones.



σ = Standard Deviation
$\pm\sigma$ = 68%
$\pm 2\sigma$ = 95%

V MEASUREMENT OF AREAS

A Methods

1 Cutting and weighing is a fairly accurate but tedious procedure. Besides the errors that may arise from thickness and moisture content of the paper, the chromatogram is destroyed. It is not satisfactory for estimating areas of overlapping peaks.

2 The planimeter is a devise whose acquisition is rarely justified. In addition to being very fatiguing, it is necessary to acquire considerable practice in its manipulation. The sensitivity of a normal planimeter is usually 10 mm^2, which is in many cases insufficient for analytical purposes.(5)

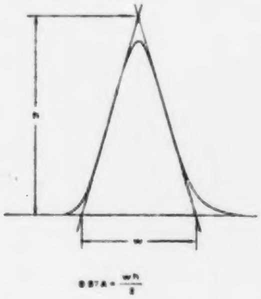
3 Integrators

a Mechanical integrators are exemplified by the Disc Integrator. This is a device which is attached to most strip chart recorders. The integrator pen tracing is displayed at the bottom of the chromatogram and the pen speed is proportional to the displacement of the recorder pen from baseline. (See triplicate runs of the nonanedecane mixture.) This is a frequently used, accurate device.

b Electronic integrators are devices which automatically print cumulative integrals of peak area. In addition to offering outstanding precision, electronic integrators automate chromatographic operations. This eliminates the time consuming necessity of the chemist keeping a watchful eye on the operation. Its biggest disadvantage is its cost.

4 Triangulation

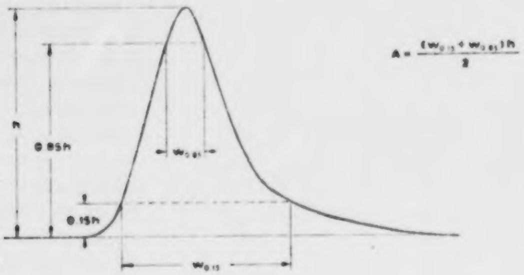
a Gaussian peaks



SAMPLE	WT. %	DENSITY	VOL. %	VOL. INJECTED, µl		W WT. INJECTED	A AREA	cm^2/ug	F CORRECTION FACTOR
		gm/cm^2		uncorr.	corr. (actual)	µg	cm^2		
Solvent	99.79	.88	99.781	1.99562	2.49453	2195.19	--	--	--
a*	.02	0.50	.035	.00070	.00087	.435	4.0	9.19	1.00
b	.03	0.75	.035	.00070	.00087	.653	6.5	9.95	1.08
c	.04	0.90	.0387	.00077	.00096	.864	7.6	8.79	.96
d	.04	0.90	.0387	.00077	.00096	.864	8.1	9.38	1.02
e	.08	1.00	.0704	.00141	.00176	1.760	15.0	8.52	.93
Total	100.00		100.00	2.00	2.50	2199.766			

*Correction factors calculated relative to "a" as standard.

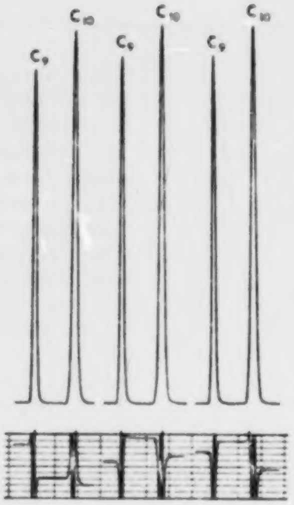
b Tailing or leading peaks, trapezoidal construction.



B Comparison of Integration Methods

Using several of the above methods on the triplicate runs of the nonanedecane mixture, the following table was obtained:

Triplicate analysis of nonane-decane mixture



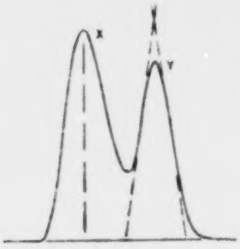
COMPARISON OF INTEGRATION METHODS

	#1	#2	#3	Avg.	σ abs	σ rel
MODEL 471 DIGITAL INTEGRATOR						
n-Nonane	39.156	39.150	39.193	39.166	0.0716	0.184%
n-Decane	60.844	60.850	60.807	60.834	0.0227	0.037%
DISC INTEGRATOR						
n-Nonane	39.33	38.97	38.91	39.07	0.22	0.56%
n-Decane	60.67	61.03	61.09	60.93	0.23	0.38%
TRIANGULATION						
n-Nonane	40.77	40.68	40.07	40.51	0.38	0.94%
n-Decane	59.23	59.32	59.93	59.49	0.39	0.66%
WEIGHING PAPER						
n-Nonane	42.58	41.73	42.83	42.38	0.58	1.37%
n-Decane	57.42	58.27	57.17	57.62	0.58	1.01%

Summary: Electronic digital integration is the most precise quantitation method; weighing paper is the least precise. Based upon this and other data in this publication, the electronic integrator give 2 to 5 times more precision than Disc Integration, 4 to 10 times more precision than triangulation, and 7 to 25 times more precision than cutting and weighing paper.

C Measurement of Overlapping Peaks

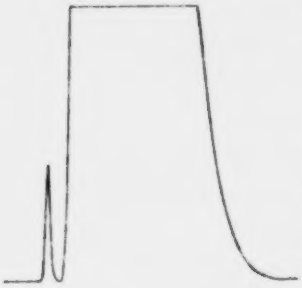
1 Components present in equal amounts.



Since broken line tangent to peak y intersects baseline after peak x has reached its maximum, then peak heights may be used. If broken line tangent to peak y intersects baseline before peak x has reached its maximum, then various known mixtures of x and y are prepared, chromatographed, and compared to the unknown x and y.

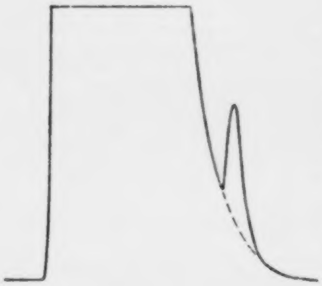
2 Trace analysis (less than 20 ppm)

a Trace component eluting before major peak.



In any trace analysis, one should choose a column and conditions which will allow the minor peak to elute before the major peak. This is easier to quantitate, and generally, peak height methods are used.

b Trace component on tail of major peak.



Assuming one has tried all conceivable columns and conditions, extend baseline as a continuation of the solvent peak and determine area. Compare this area with a synthetic mixture. Also, one can "spike" the sample with known amount of the trace component and compare area before and after.

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5 Lewin, S. Z. Journal of Chemical Education, 41, No. 4, A235-A259, April 1964.

This outline was prepared by E. J. Bonelli, Applications Manager, Varian Aerograph Company, Walnut Creek, California.

Descriptors: Chemical Analysis, Chromatography, Gas Chromatography, Instrumentation, Measurement, Separation Techniques, Water Analysis

RECENT DEVELOPMENTS IN THE IDENTIFICATION OF OILS BY INSTRUMENTAL ANALYSES

Part 1

I INTRODUCTION

- A Health Hazards of Oil
- B Analytical Procedures
- C Need

II EXPERIMENTAL

- A Sampling
- B Preparation
- C Variations

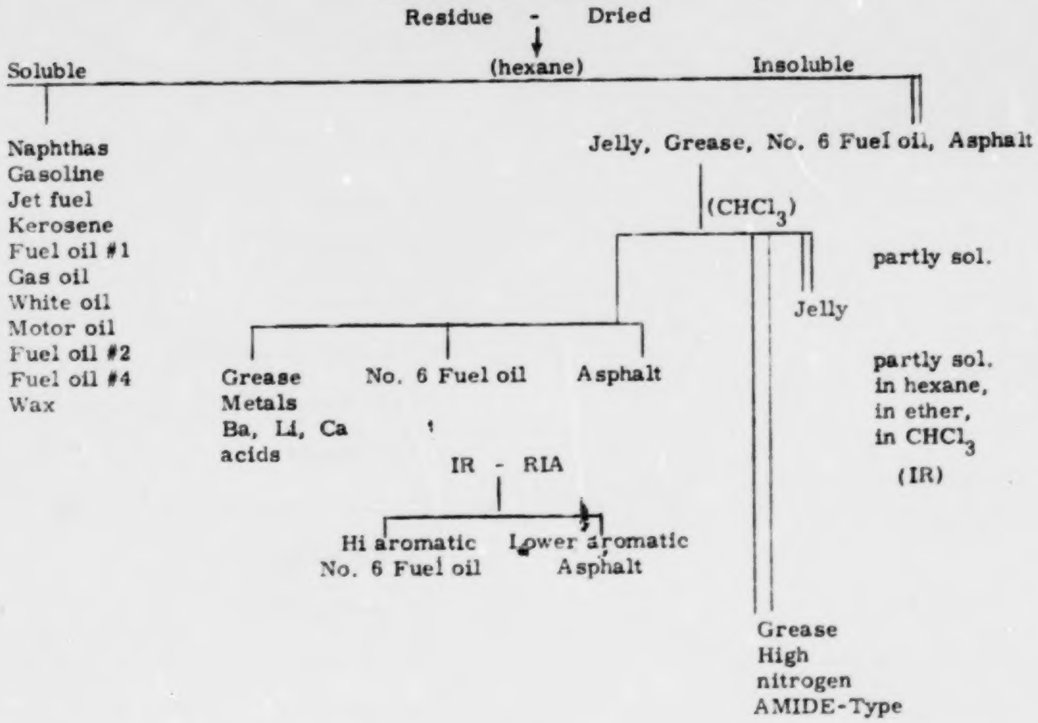
III ANALYSES

- A Characterization Scheme
- B Solubility
- C Density
- D Initial B.P., final B.P.
- E Infrared Analyses
  - 1 Spectra
  - 2 Ratios
  - 3 Mixtures
- F Gas Chromatography
- G Case Histories
  - 1 Asphalts
  - 2 Heavy residual oils
  - 3 Heater oil
  - 4 Lubricating oil
  - 5 Xylenes
  - 6 Gasolines

This outline was prepared by F. K. Kawahara, Organic Chemist, Analytical Quality Control Laboratory, 1014 Broadway, Cincinnati, OH 45202.

Descriptors: Chemical Analysis, Chromatography, Gas Chromatography, Infrared Radiation, Instrumentation, Oil, Oil Wastes, Water Analysis

Characterization Scheme


Residue - Dried
(hexane)
Soluble
Insoluble
Naphthas
Gasoline
Jet fuel
Kerosene
Fuel oil #1
Gas oil
White oil
Motor oil
Fuel oil #2
Fuel oil #4
Wax
Jelly, Grease, No. 6 Fuel oil, Asphalt
(CHCl3)
partly sol.
Jelly
partly sol.
in hexane,
in ether,
in CHCl3
(IR)
Grease
Metals
Ba, Li, Ca
acids
No. 6 Fuel oil
Asphalt
IR - RIA
Hi aromatic
No. 6 Fuel oil
Lower aromatic
Asphalt
Grease
High
nitrogen
AMIDE-Type

Residue - Dried

↓

Soluble (hexane)

↓

	I.B.P. - e.P.	API - gravity	Comment
High gravity naphtha	95° - 206° F	45 - 75	
Gasoline	96° - 408° F	58 - 62	
Low gravity naphtha	160° - 410° F	30 - 52	
Jet fuel	100° - 500° F	40 - 55	
Kerosene	355° - 575° F	40 - 46	
Fuel oil #1	360° - 625° F	> 35	k. vis.
Gas oil	400° - 800° F	30 - 33	
White oil	-	29 - 32	I.R. paraffin
Motor oil	-	24 - 30	Zn, P, S
Fuel oil #2	370° - 625° F (90%)	> 26	k. vis.
Fuel oil #4	420° - 683° F	9 - 36	k. vis.
Wax	MP = 145° - 190° F	34 - 39	Solid - IR paraffinic

ANALYSES OF OIL SLICKS

Part 2

I INTRODUCTION

A Foreword

Oil pollution has become a major problem in the coastal and surface waters of the United States and other countries. Discharges and spills from offshore industrial plants and commercial ships impart unpleasant taste and odor to potable water and to game fish and produce unsightly conditions which ruin beaches and recreational facilities. As predicted, carcinogens present in heavy oils, such as lube oils, heavy residual oils and asphalts, have already been found in flora and probably will be found in fish, etc.

The urgency of these problems requires that procedures for detection, characterization, and identification of the oil pollutants be developed to forewarn of the impending health hazards and to establish responsibility for violations of water quality standards.

In some cases, comparative examination of the infrared spectra of the oil slick collected from surface water with those of the suspect oil may be sufficient. In many cases eye witnesses are not available. Chemical analyses must be made to facilitate identifying the source of pollution. Thus a definite knowledge of the composition and character of the oil slick is vital as characterization is the preliminary step toward identification of the source of the pollutant of unknown origin. This procedure will aid in establishing the type of oil product and also in the identification of the source of the oil pollutant.

B Procedure

The complete procedure involves collection, extraction, drying, concentration and analyses.

C Equipment and Materials

Although conditions of heavier petroleum samples may vary, all are extracted with analytical grade dichloromethane (B.P. 40°C).

Dried samples of petroleum products and pollutants may be analyzed with the Perkin-Elmer 137 Infracord Spectrophotometer* that may be operated at slow-scan speed with a slit opening of 25 mm.

Salt prisms are polished on felt with the aid of rouge (Barnesite) mixed with ethanol.

For solvent removal, a commercially-available rotating vacuum flask evaporator or an all glass vacuum distillation system consisting of flask, Claisen head, condenser and receiver is suitable. Either of the two is attached to a dry ice trap leading to a mechanical vacuum pump or a building vacuum line.

II PROCEDURE

A Collection

Collect the oily material from the surface of the water with the aid of a large household mop with a ringer attachment. Before use, rinse the sponge thoroughly with dichloromethane. Pass the mop through the oil pool, squeeze and transfer by means of a widemouth funnel the absorbed oil into the collecting jar. If a mop is not available, sample the oil slick with aid of a widemouth bottle and transfer the contents to a larger bottle. Where possible, obtain reference samples of oil product from vessels and/or shore facilities.

B Preservation of Sample

Protect the samples containing the water and oil against autoxidation and other chemical conversions by removal of air and exclusion of light. Add a tiny piece of dry ice to the sample. When the effervescing has stopped, seal the jar with a teflon lined cap. Preserve the samples in the refrigerator whenever possible. When nitrogen or an inert gas is not available, carefully fill with water the sample bottle to the top in order to displace much of the air.

C Sample Preparation for Analyses

To prepare a petroleum sample for analysis, extract the water sample with purified dichloromethane. Add 175 ml of solvent to a 500 ml separatory funnel. Add a portion amounting to about one-half to five grams of petroleum pollutant together with the large volume of sample water. Tilt the separatory funnel back and forth for one minute to extract the product. Set the funnel and contents on a ring stand placed under a well-ventilated hood. Draw the lower extract off and collect in a suitable Erlenmeyer flask. Repeat the operation, if necessary.

Place the combined extracts in the separatory funnel. Add 5 ml of distilled water and wash the extract by shaking gently. Allow the extract phase to separate from the water phase. Permit the lower solvent layer to pass dropwise through 5 grams of anhydrous sodium sulfate held in a No. 12 folded filter paper seated on a filtering funnel with a 75 mm diameter and 75 mm stem. Collect the filtered, dried pollutant extract in a 300 ml round bottom flask equipped with a standard tapered 24/40 ground glass joint.

NOTE: If the petroleum pollutant is considered to be more volatile than kerosene, centrifuge the sample if sufficient petroleum material is obtainable. Use a special syringe for the removal of the top phase. Transfer the contents from the needle syringe to a micro, conical, graduated, centrifuge tube of 2.5 ml capacity. Add a few grains of anhydrous sodium sulfate to dry the transferred light petroleum pollutant.

D Concentration

Attach to a rotating-type, vacuum evaporator, the round bottom flask with dried dichloromethane extract of the petroleum pollutant. If the amount of residue is small, rinse thoroughly the sides of the flask with about 5 to 10 ml of dichloromethane. Transfer the contents to a 20 ml conical, graduated centrifuge tube. Evaporate off the transferring solvent. If upon infrared analyses bands appear at 7.85 μ and 13.55 μ, continue drying until they no longer appear.

E Solubility Studies

From the thoroughly dried residue, free of water and extracting solvent, take about 0.2 gram portion and place into a 20 ml vial or test tube. Add seven (7) ml of hexane and stir the mixture with a glass rod. Observe whether the transferred residue is entirely soluble in hexane. Repeat the test to determine the solubility in seven (7) ml of diethyl ether. Determine the solubility in chloroform.

If the material is soluble in each of the three solvents, assume that the unidentified material is one of the following: light or heavy naphtha, kerosene, gas oil, white oil, cutting oil, motor oil, diesel oil or jet fuel. If the material is insoluble in hexane and in ether, but readily soluble in chloroform, assume that it is a grease, a heavy residual oil (No. 6, No. 5 or No. 4) or an asphalt. Refer to Table 1 on Solubility of Petroleum Products.

TABLE 1

SOLUBILITY OF PETROLEUM PRODUCTS

Product	Hexane	Ether	Chloroform
Light naphtha	VS	VS	VS
Heavy naphtha	VS	VS	VS
Gasoline	VS	VS	VS
Jet fuel	VS	VS	VS
Kerosene	VS	VS	VS
Gas oil	VS	VS	VS
Diesel oil	VS	VS	VS
White oil	VS	VS	VS
Cutting oil	S	S	S
Motor oil	S	S	S
Paraffin wax	S	S	S
White petroleum jelly	PS	PS	PS
Grease	I	I	S
Residual fuel oil	I	I	S
Asphalt feed stock	I	I	S

F Determination of API Gravity

Measure the density of the oil by the pycnometric methods, ASTM D-941 or ASTM D-1217. Weigh the dry, empty pycnometer (We). Weigh the same pycnometer when it is filled with oil (Wo). Empty and dry the pycnometer. Weigh the pycnometer when it is filled with water (Ww). Calculate the density, using the following formula:

$$\text{Density} = \frac{\text{Wo} - \text{We}}{\text{Ww} - \text{We}}$$

Compare the density with those given for petroleum products listed in Table 2.

III CHARACTERIZATION

A Chromatographic Analyses

For the identity of classified, volatile petroleum products, such as naphtha, gasoline, jet fuel and kerosene, analyze by means of flame ionization detection gas chromatograph the dried recovered residual material.

B Infrared Analyses

As greases, heavy residual fuel oil, and asphalt are less volatile, compare with the reference spectrum the unidentified spectrum obtained by infrared spectrophotometry. (If the unknown has been exposed to air and sunlight, consider possible changes due to peroxidation. Carbonyls will appear at about 5.75 μ to 5.85 μ and ethers at about 8.7 to 9.4 μ.) For viscous oils and asphalt, place the material between two salt plates. Place the plates on the crystal holder which may be detached from the instrument. Obtain the infrared spectrum after calibrating the instrument using a polystyrene form. For motor oils, use a cell. Obtain spectra also for reference and industrial comparison samples.

C Ratios of Infrared Absorbance

Measure the infrared absorbances at the following frequencies:

$3050^{\text{cm}^{-1}}$, $2925^{\text{cm}^{-1}}$, $1600^{\text{cm}^{-1}}$,

$1375^{\text{cm}^{-1}}$, $810^{\text{cm}^{-1}}$, $720^{\text{cm}^{-1}}$

Determine the length of the vertical line, which intersects the line tangential to the two proximate inflections. Consider the ratio of intensity of the infrared absorption at one frequency to that intensity of the absorption at another frequency. Calculate the following ratios and compare with those for the products listed in Table 3.

TABLE 2

TABLE OF DENSITY PROPERTIES OF PETROLEUM PRODUCTS

Material	Density	API Gravity
Crude	0.857 - 0.976	13.5 - 33.5
High gravity naphtha	0.684 - 0.802	45 - 75
Low gravity naphtha	0.868 - 0.877	30 - 53
Gasoline	0.732 - 0.747	58 - 62
Jet fuel	0.759 - 0.825	40 - 55
Kerosene	0.797 - 0.825	40 - 46
Gas oil	0.859 - 0.877	30 - 33
White oil	0.866 - 0.882	29 - 32
Motor oil	0.877 - 0.909	24 - 30
Fuel oil #1	>0.849	35
Fuel oil #2	0.898	26
Fuel oil #4	0.844 - 1.006	9 - 36
Fuel oil #5	0.909 - 1.025	6.5 - 24
Fuel oil #6	0.917 - 1.101	-3 to 23
Asphalt	0.947 - 1.147	-8 - 18

Establish the characterization of the petroleum product by comparative evaluation of the two ratios.

$$\frac{810^{cm-1}}{1375^{cm-1}} \text{ versus } \frac{810^{cm-1}}{720^{cm-1}}$$

Plot the ratio values on graph paper similar to Figure 1 and determine from the commercial asphalt-fuel oil line, $y = 0.13x + 0.13$, whether the point of the unknown lies in the asphaltic of in the heavy residual oil classification. Make a similar examination of the reference sample that will be compared to the unknown.

Asphalts are represented by values clustered between 0.6 and 1.3 for the $\frac{810^{cm-1}}{720^{cm-1}}$ ratio while heavy residual oils lie at points greater than 1.30 for this ratio.

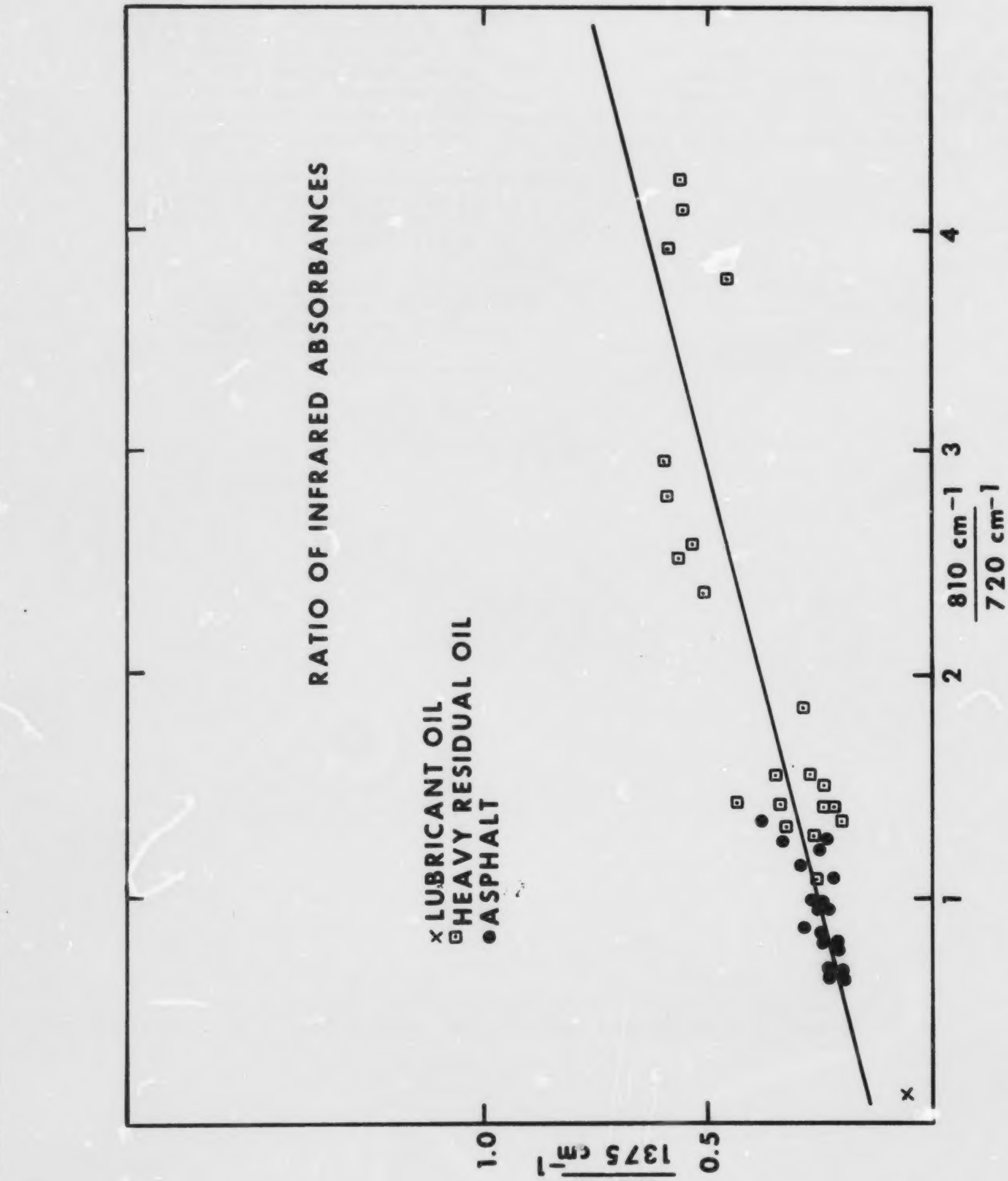
IV IDENTIFICATION

For identification purposes, determine whether the spectra of the collected oil slick and the suspect oil match along the entire spectral region between 2.5 μ and 15 μ. A match will establish the responsible party.

TABLE 3

RATIOS OF INFRARED ABSORBENCES OF COMMERCIAL ASPHALTS, NO. 6 FUEL OILS, AND LUBE OILS

RIA	Asphalt	No. 6 Fuel Oil	Lube Oil
$\frac{720^{cm-1}}{1375^{cm-1}}$	0.23	0.20	0.48
$\frac{3050^{cm-1}}{2925^{cm-1}}$	0.22	0.28	0.20
$\frac{810^{cm-1}}{1375^{cm-1}}$	0.25	0.40	0.06
$\frac{810^{cm-1}}{720^{cm-1}}$	0.94	2.18	0.12
$\frac{1600^{cm-1}}{1375^{cm-1}}$	0.54	0.60	0.11
$\frac{1600^{cm-1}}{720^{cm-1}}$	2.05	3.27	0.24


RATIO OF INFRARED ABSORBANCES
× LUBRICANT OIL
⊡ HEAVY RESIDUAL OIL
● ASPHALT
1.0
0.5
810 cm⁻¹ / 1375 cm⁻¹
1
2
3
4
810 cm⁻¹ / 720 cm⁻¹

V ERROR

The error is estimated to be about ± 10%. Deviations may be greatly minimized by averaging the data from four spectra at thickness of oil adjusted so that the transmission reading is 85% to 98% of full scale at 3050^{cm-1}.

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This outline was prepared by F. K. Kawahara, Organic Chemist, Analytical Quality Control Laboratory, 1014 Broadway, Cincinnati, OH 45202.

Mention of the products and manufacturers is for identification only and does not imply endorsement by the Federal Water Pollution Control Administration of the U.S. Department of the Interior.

INFRARED INSTRUMENTATION

I THEORY

A The Effect of Infrared Radiation on the Molecule

1. All molecules are made up of atoms connected by chemical bonds which act very much like springs.
2. Atoms or atomic groups are in continuous motion with respect to each other. Each molecule has specific vibrational frequencies for these motions.
3. A molecule which has the same vibrational frequency as the infrared radiation striking it will absorb that energy.
4. If the radiant frequency differs from the characteristic frequencies of the molecule, the radiation passes through the molecule undiminished.
5. The characteristic frequencies for particular molecules are determined by the masses of their atoms, their spatial geometry and the strength of their connecting bonds.

B Measuring Infrared Radiation

1. The infrared spectrum gives basic information about the molecular structure of a compound.
2. Organic chemicals are largely made up of different combinations of atomic building blocks called functional groups: $-OH$, $-NH_2$, $-CH_3$, $-CO$, $-CN$, $-\overset{|}{\underset{|}{C}}-O-\overset{|}{\underset{|}{C}}-$, $-COOH$.
3. These subgroups have characteristic absorption bands in certain parts of the infrared spectrum.
4. Spectra correlation charts provide a key to the location of characteristic absorption bands of the most common functional groups.

II THE INSTRUMENT

A Source

1. A length of ceramic tubing heated to about 1200°C
2. It produces a continuous spectrum of radiant energy, most of which falls within 2. 5 to 15 micron region.

B Monochromator

1. It disperses the radiation into its component wavelengths.
2. Selects the particular wavelength interval transmitted to the detector.
3. Maintains, by varying slit widths, approximately constant energy through the slits to the detector.

C Wavelength

1. A mirrors rotational position determines the particular wavelength which emerges from the slit.
2. Linkage with the recorder drum shaft insures that the wavelength scale on the drum corresponds exactly to the wavelength selected.

D Resolution

1. A NaCl prism provides the best combination of dispersion and transmission in the operating range.
2. The sealed and desiccated monochromator protects the prism from moisture and dust.

E Thermocouple

1. A high sensitivity thermocouple consists of a blackened gold leaf target welded to two pins.

2 Temperature differences at the junctions of the leaf and pins set up a thermoelectric potential which is utilized.

F Optical Attenuator

1 By subtracting energy from the reference beam, the attenuator makes the amount of energy in the reference beam precisely the same as that in the sample beam.

2 This permits the pen to record directly in terms of sample transmittance.

G Instrument Performance

1 Scanning time

Twelve minutes for total scan, automatically programmed for constant rate per spectral slit width for constant accuracy over the wavelength range.

2 Accuracy

a Abscissa ± 0.03μ, ordinate ± 1%T.

b Less than 3% scattered light at 14.7μ and less than 1% from 2.5μ to 14μ.

III MICRO INFRARED TECHNIQUE

A Solution Technique

1 The technique most reliable for quantitative measurements in infrared region is the solution technique.

2 One drawback to this technique is the scarcity of suitable solvents with sufficient areas of transparency.

3 Carbon disulfide CS_2 is the most valuable of these because of its transparency in the "finger print" region of the infrared from 1,350 to 650 cm^{-1} (7.4μ to 15). Carbon tetrachloride is a useful solvent from 4,000 to 1,650 cm^{-1} (2.5μ to 6.0μ).

4 The sample is dissolved in 0.3 ml of solvent. The solution is then transferred to a 5 mm energy-path cavity cell by a hypodermic syringe. A matching cavity cell is used for solvent compensation.

5 With this technique, sensitivities down to 5-10 μg of strongly absorbing compounds are achieved without resorting to scale expansion.

B The Potassium Bromide KBr Pellet Technique

1 Since KBr is transparent throughout the commonly used portion of the infrared region, the entire infrared region is available as contrasted to the use of solvents.

2 A 6X beam-condenser is used to increase the energy transmitted through the pellet.

3 By this procedure a reliable sensitivity of about 5μg can easily be attained without scale expansion.

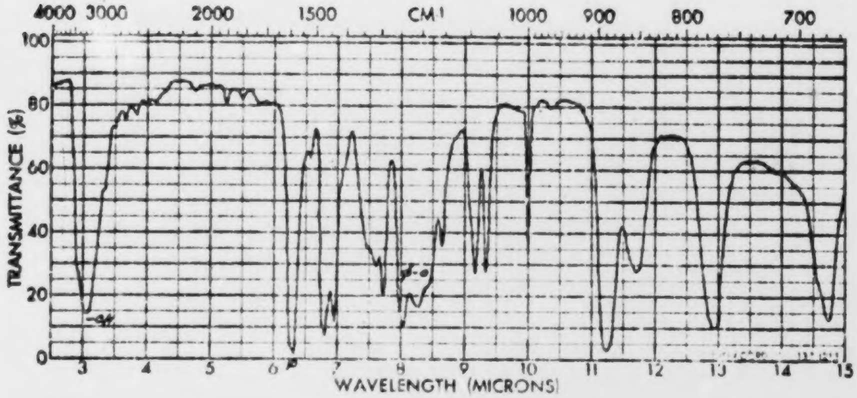
REFERENCES

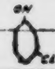
1 Blinn, R.C. Infrared technique useful in residue chemistry, AOAC. Vol. 48, 1009-1017. 1965.

2 InfraCord, Perkin-Elmer spectrophotometer model 137-B Brochure, 1965.

This outline was prepared by J.W. Mandia, Chemist, formerly with National Training Center, MDS, WPO, EPA, Cincinnati, OH 45268.

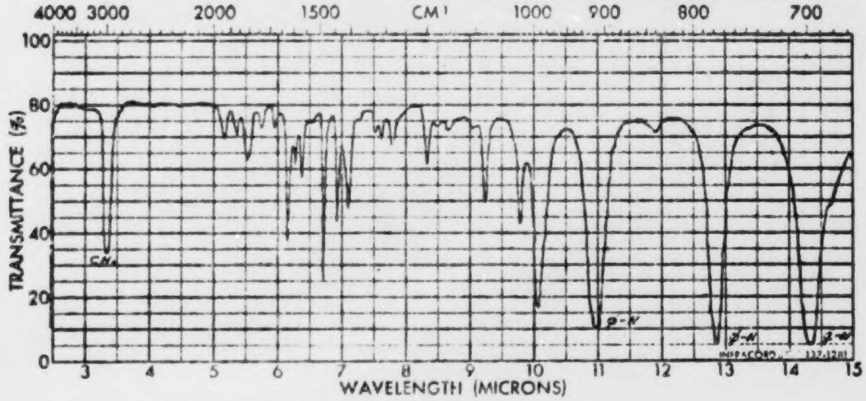
Descriptors: Chemical Analysis, Infrared Radiation, Instrumentation, Water Analysis



SPECTRUM NO. 11	ORIGIN	LEGEND	REMARKS
SAMPLE		1.	
M-CHLOROPHENOL	PURITY ACS	2.	
	PHASE LIQUID	DATE 3-24-59	
	THICKNESS SNDWCH	OPERATOR DGB	

SPECTRUM NO.____ SAMPLE

THE PERKIN-ELMER CORPORATION, NORWALK, CONN.



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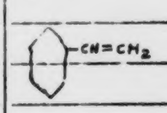
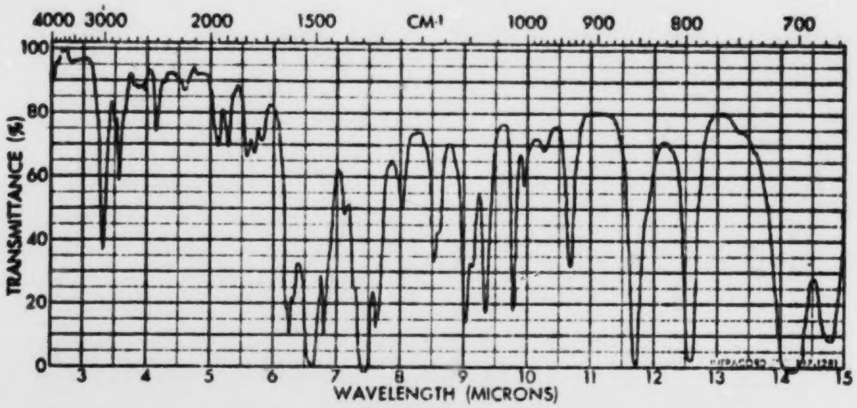
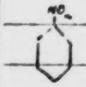
SAMPLE		1.	
STYRENE	PURITY ??	2.	
	PHASE LIQUID	DATE 4-14-59	
	THICKNESS SNDWCH	OPERATOR DGB	

SPECTRUM NO.____ SAMPLE

CH.OTS 1eh.1 - 6.6.5

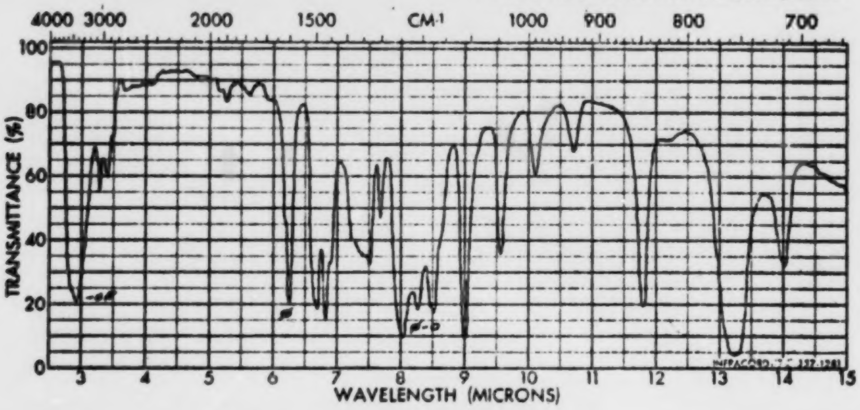
THE PERKIN-ELMER CORPORATION, NORWALK, CONN.

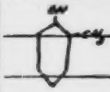


SPECTRUM NO. 9	ORIGIN	LEGEND	REMARKS 
SAMPLE		1.	
NITROBENZENE	PURITY ACS	2.	
	PHASE LIQUID	DATE 3-4-59	
	THICKNESS 0.25 MM	OPERATOR DJB	

SPECTRUM NO.___ SAMPLE

THE PERKIN-ELMER CORPORATION, NORWALK, CONN.

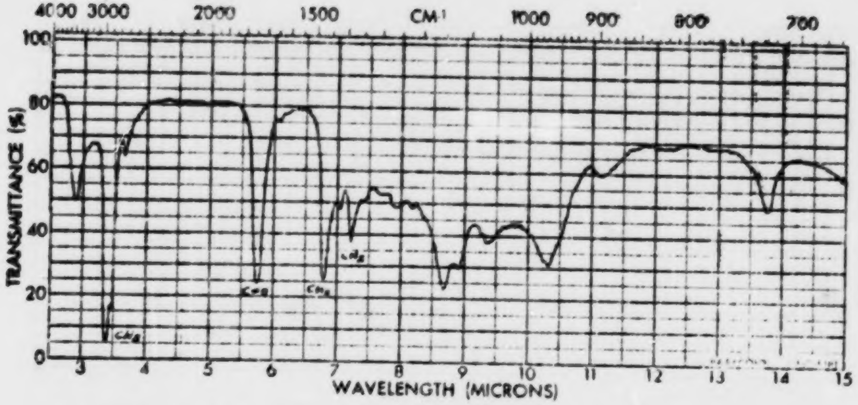


SPECTRUM NO. 10	ORIGIN	LEGEND	REMARKS 
SAMPLE		1.	
O-CRESOL	PURITY ACS	2.	
	PHASE LIQUID	DATE 3-10-59	
	THICKNESS 0.2 MM.	OPERATOR DJB	

SPECTRUM NO.___ SAMPLE

GR.OTS.lab.3.b.59

THE PERKIN-ELMER CORPORATION, NORWALK, CONN



SPECTRUM NO. 7
SAMPLE
OCTANALDEHYDE

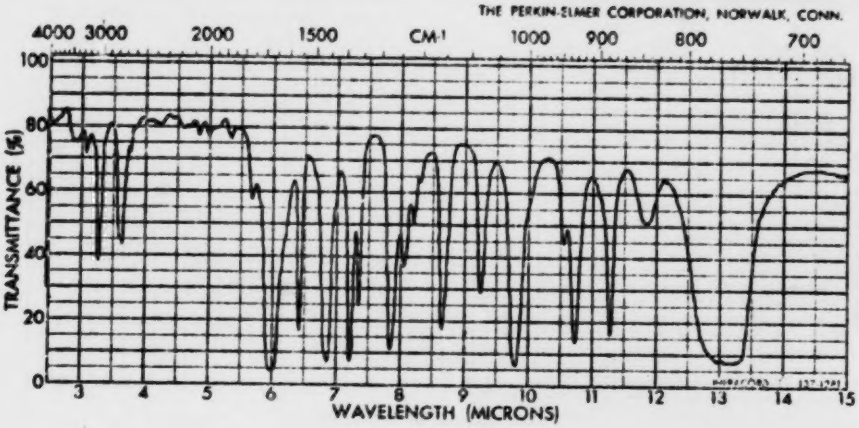
ORIGIN
PURITY ??
PHASE Liquid
THICKNESS SNDWCH

LEGEND
2
DATE 4-13-59
OPERATOR DGB

REMARKS
$CH_3CH_2CH_2CH_2-CH_2CH_2CH_2CHO$

SPECTRUM NO.
SAMPLE

THE PERKIN-ELMER CORPORATION, NORWALK, CONN.

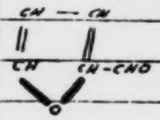


SPECTRUM NO. 8
SAMPLE
FURFURAL

ORIGIN
PURITY ??
PHASE Liquid
THICKNESS SNDWCH

LEGEND
1.
2.
DATE 3/23/59
OPERATOR DGB

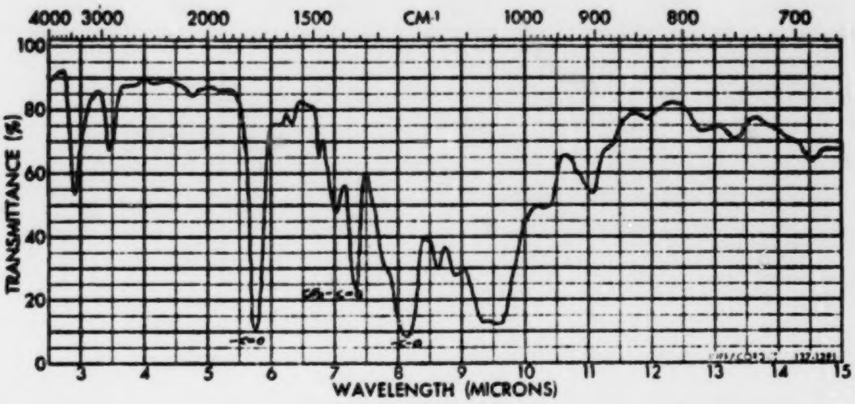
REMARKS



SPECTRUM NO.
SAMPLE

CH.ITS.1ab.4.4.59

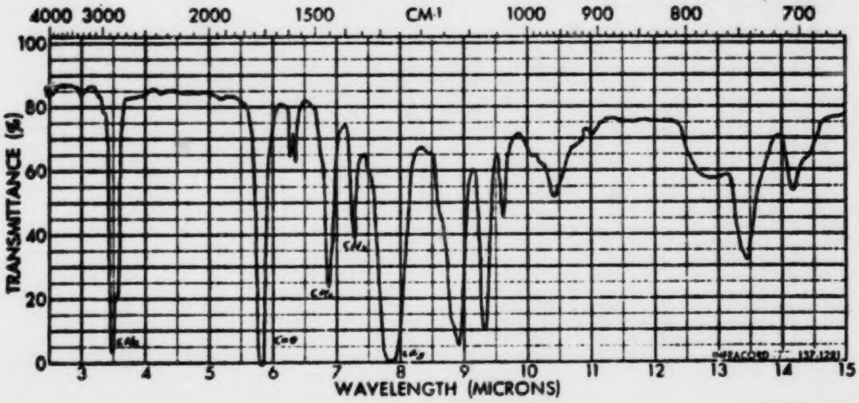
THE PERKIN-ELMER CORPORATION, NORWALK, CONN.

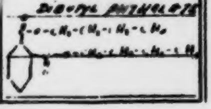


SPECTRUM NO. 5	ORIGIN	LEGEND	REMARKS
SAMPLE		1.	
Acetate	PURITY ??	2.	$CH_3-\overset{O}{\overset{\parallel}{C}}-O-$
	PHASE GEL	DATE 4-1-59	
	THICKNESS SMEAR	OPERATOR DGB	

SPECTRUM NO.____ SAMPLE____

THE PERKIN-ELMER CORPORATION, NORWALK, CONN.

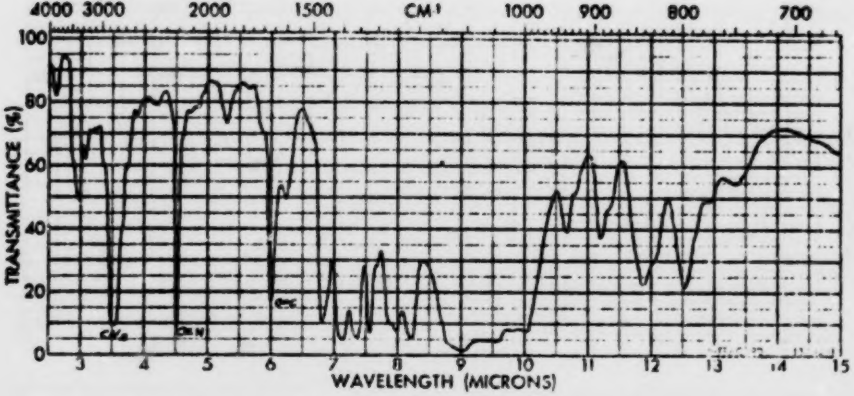


SPECTRUM NO. 6	ORIGIN	LEGEND	REMARKS
SAMPLE		1.	Principally
Tygon Plasticizer	PURITY ??	2.	Dibutyl phthalate
	PHASE GEL	DATE 4-6-59	 $\overset{O}{C}-O-CH_2-CH_2-CH_2-CH_3$ $\underset{O}{C}-O-CH_2-CH_2-CH_2-CH_3$
	THICKNESS SMEAR	OPERATOR DGB	

SPECTRUM NO.____ SAMPLE____

CW.OTS.lab.3.4.59

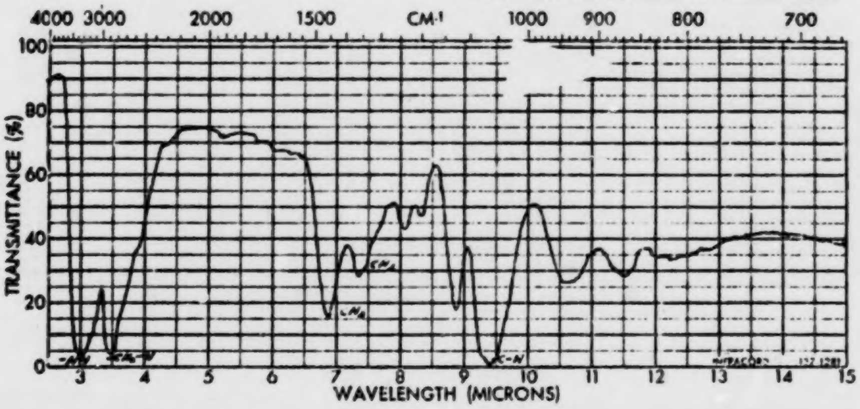
THE PERKIN-ELMER CORPORATION, NORWALK, CONN.



SPECTRUM NO. 7	ORIGIN	LEGEND	REMARKS
SAMPLE		1.	
oxydipropionitrile	PURITY ACS	2.	$NC-CH_2-CH_2-O-CH_2-CH_2-C\equiv N$
	PHASE Liquid	DATE 4-3-59	
	THICKNESS 0.2 mm	OPERATOR DGB	

SPECTRUM NO.
SAMPLE

THE PERKIN-ELMER CORPORATION, NORWALK, CONN.

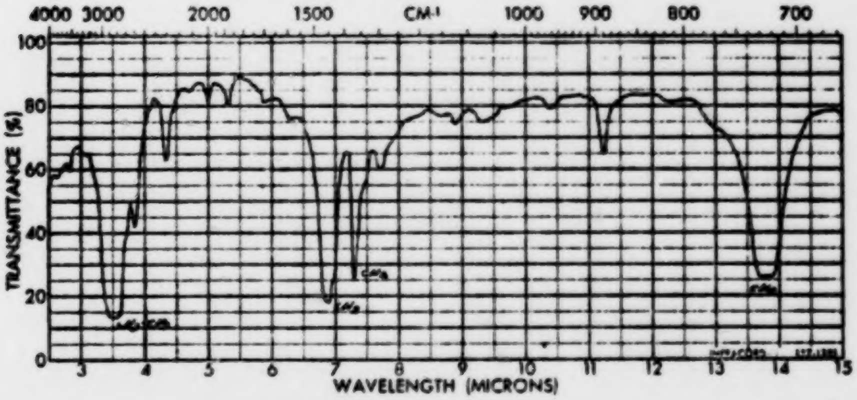


SPECTRUM NO. 89	ORIGIN	LEGEND	REMARKS
SAMPLE (4)		1.	
Diethanolamine	PURITY ACS	2.	$HO-CH_2-CH_2-NH-CH_2-CH_2-OH$
	PHASE Liquid	DATE 4-2-59	
	THICKNESS SNDWCH	OPERATOR DGD	

SPECTRUM NO. 89
SAMPLE Diethanolamine

GR.OTS.lab.9.L.59

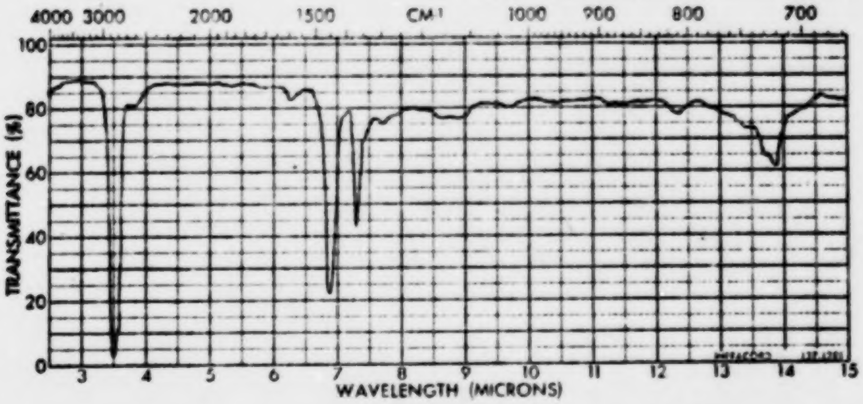
THE PERKIN-ELMER CORPORATION, NORWALK, CONN.



SPECTRUM NO. 1	ORIGIN	LEGEND	REMARKS
SAMPLE		1.	
Paraffin waxes	PURITY [illegible]	2.	$-CH_2-CH_2-CH_2-$
	PHASE Gel	DATE 2-11-59	
	THICKNESS smear	OPERATOR DGB	

SPECTRUM NO. ___ SAMPLE

THE PERKIN-ELMER CORPORATION, NORWALK, CONN.

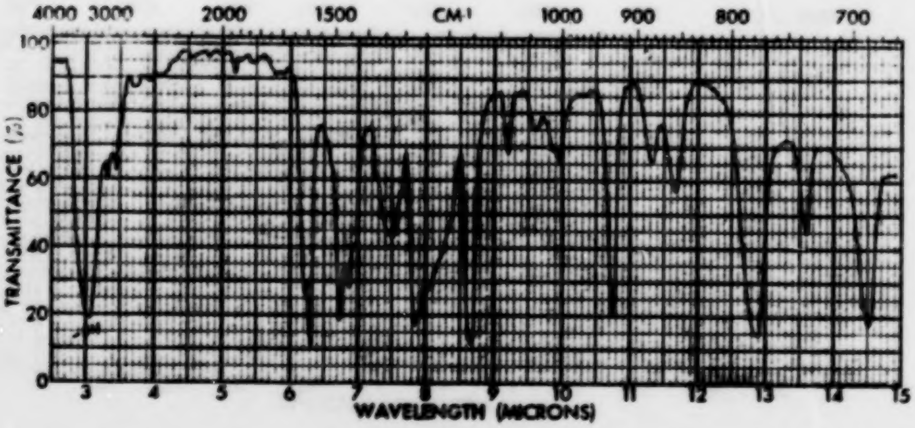


SPECTRUM NO. 2	ORIGIN	LEGEND	REMARKS
SAMPLE		1.	
Petroleum residue	PURITY —	2.	
	PHASE Gel	DATE 2-11-59	
	THICKNESS smear	OPERATOR DGB	

SPECTRUM NO. ___ SAMPLE

CH.UTS.1ab.1.6.59

THE PERKIN-ELMER CORPORATION, NORWALK, CONN.



SAMPLE NO. 13

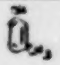
SOURCE PURE m-CRESOL

LOCATION

DATE SAMPLED

TYPE ALDRICH

REMARKS



PHASE LIQUID

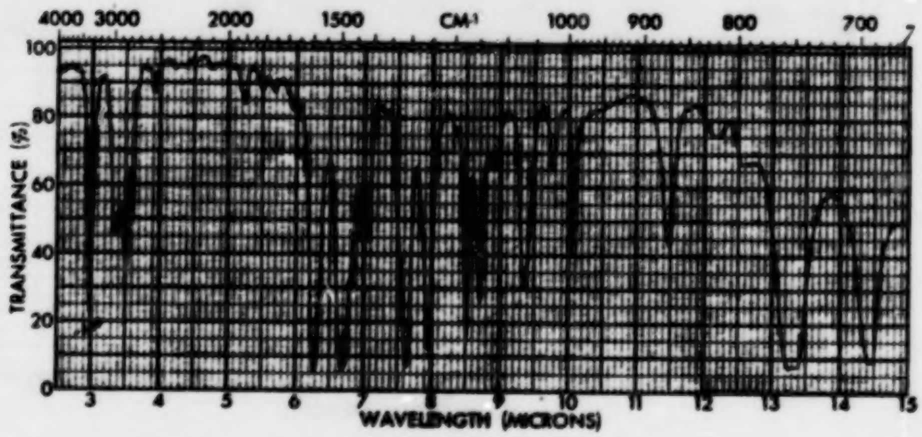
OPERATOR [illegible]

DATE 3-14-63

SAMPLE NO.

SOURCE

LOCATION



SAMPLE NO. 14

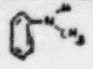
SOURCE N-METHYL ANILINE

LOCATION

DATE SAMPLED

TYPE ALDRICH

REMARKS



PHASE LIQUID

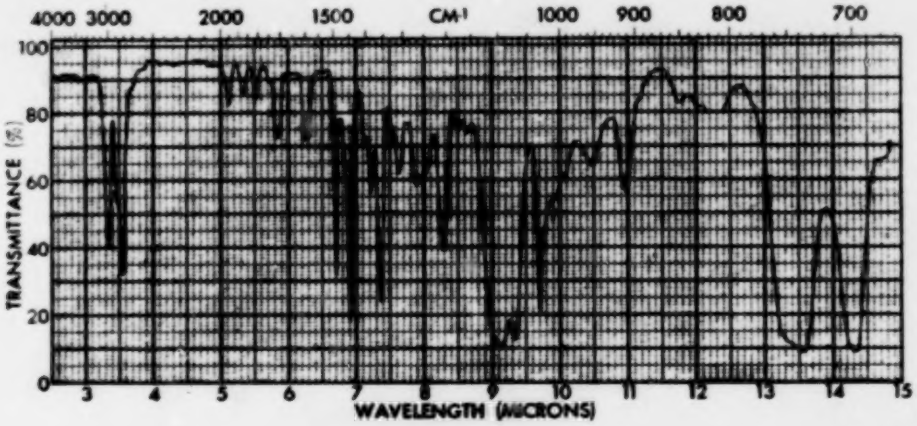
OPERATOR [illegible]

DATE 3-14-63

SAMPLE NO.

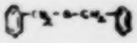
SOURCE

LOCATION



SAMPLE NO. 15
SOURCE PURE BENZYL ETHER
LOCATION
DATE SAMPLED
TYPE SANDWICH

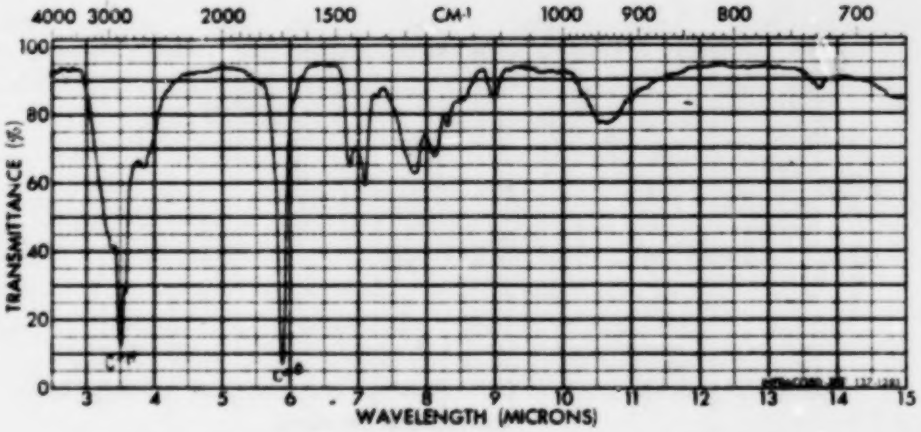
REMARKS

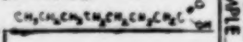


$C_6H_5-CH_2-O-CH_2-C_6H_5$

PHASE LIQUID
OPERATOR BAP
DATE 3-6-63

SAMPLE NO.
SOURCE
LOCATION



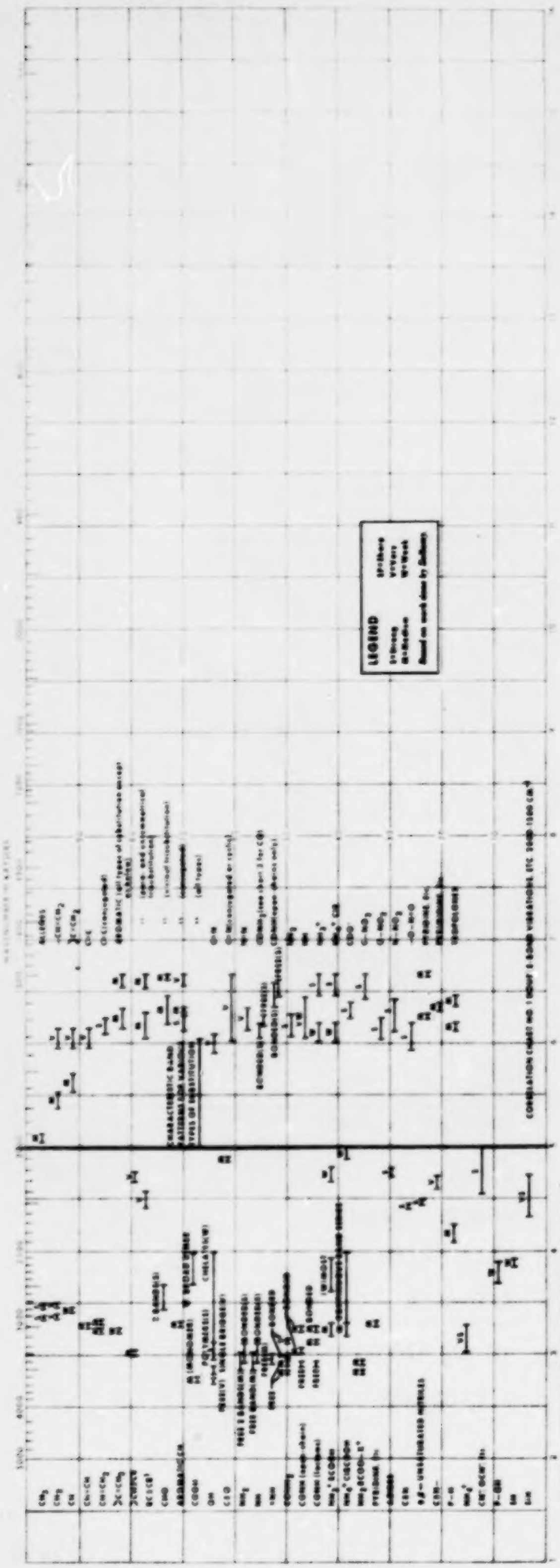
SPECTRUM NO. 16	ORIGIN	LEGEND	REMARKS
SAMPLE CAPRYLIC ACID		1	 $CH_3CH_2CH_2CH_2CH_2CH_2CH_2C\begin{smallmatrix}\diagup O\\ \diagdown OH\end{smallmatrix}$
	PURITY PURE	2	
	PHASE LIQUID	DATE 2-11-63	
	THICKNESS NEAT	OPERATOR BAP	

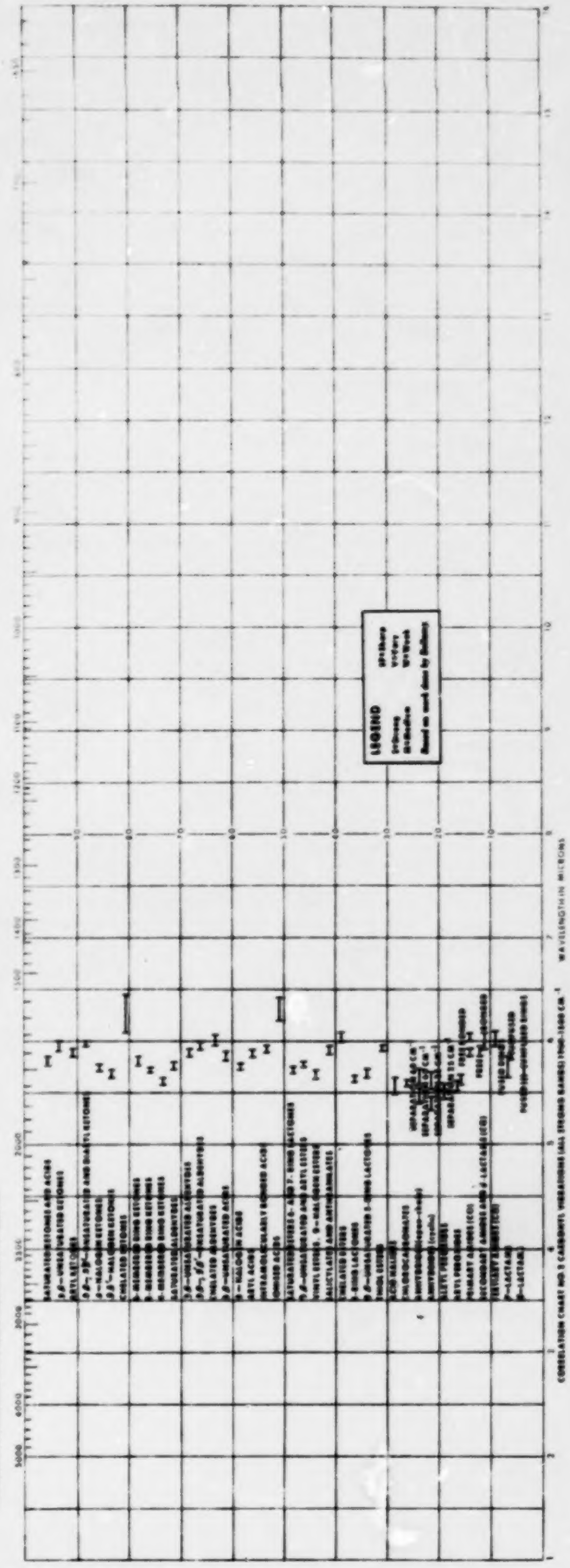
SPECTRUM NO.
SAMPLE

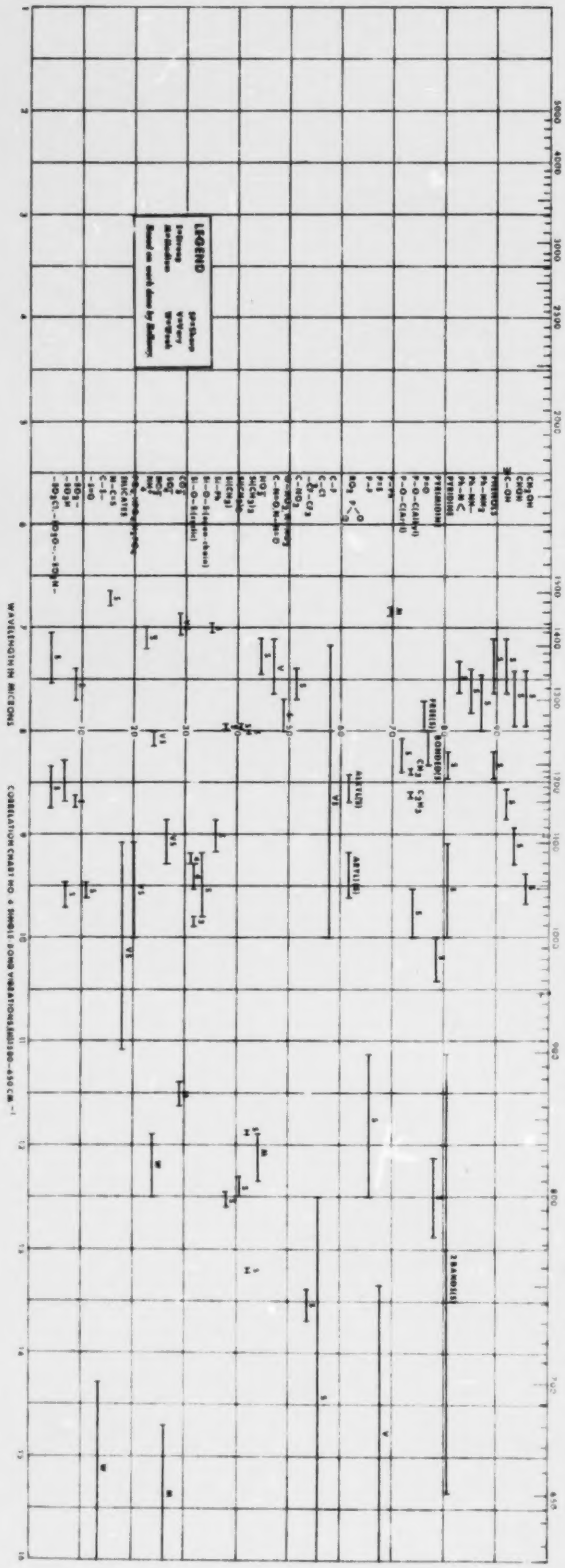
THE PERKIN-ELMER CORPORATION, NORWALK, CONN.

DATA SHEET - ORGANIC LABORATORY

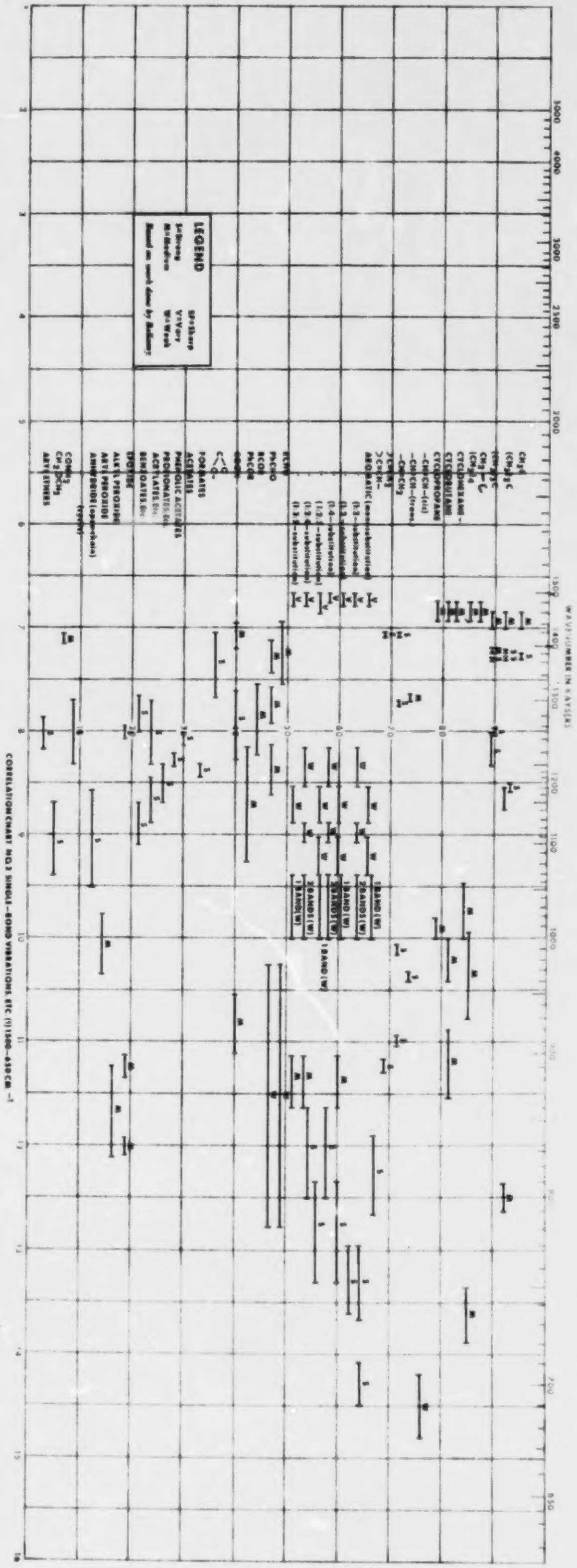
SOLUBILITY SEPARATION		CHROMATOGRAPHIC SEPARATION OF NEUTRALS	
WEIGHTS	REMARKS	WEIGHTS	REMARKS
ORIGINAL SAMPLE __________ GM		ALIQUOT USED __________ __________ GM	
BASES __________ __________ GM		ALIPHATICS __________ __________ GM	
STRONG ACIDS __________ __________ GM		AROMATICS __________ __________ GM	
WEAK ACIDS __________ __________ GM		OXYS __________ __________ GM	
NEUTRALS __________ __________ GM		SUMMARY OF DATA BASES __________ GM STRONG ACIDS __________ WEAK ACIDS __________ NEUTRALS __________ TOTAL RECOVERED __________ GM % RECOVERY __________ %	


LEGEND
WAVELENGTH IN MICRONS
HYDROGEN STRETCHING AND TRIPLE-BOND VIBRATIONS 3750-2000 CM-1


LEGEND
WAVELENGTH IN MICRONS
CORRELATION CHART NO. 2 CARBONYL VIBRATIONS (ALL STRONG BANDS) 1900-1500 CM-1



CORRELATION CHART NO. 4 SINGLE BOND VIBRATIONS (III) 1500–650 CM^{-1}



CORRELATION CHART NO. 3 SINGLE-BOND VIBRATIONS, ETC (II) 1500–650 CM^{-1}

ANALYSIS OF FISH TISSUE
FOR ORGANO CHLORINE COMPOUNDS

I OBJECTIVE

Upon completion of this laboratory, the trainee will be able to extract, partition, cleanup and identify organo chlorine compounds in fish tissue. The student should be able to repeat the procedure in his own laboratory by using the printed laboratory procedure.

II REAGENTS

A Hexane, Burdick and Jackson, BP 68-69° C (redistilled)

B Acetonitrile, Burdick and Jackson, BP 80 - 2° C (redistill with 1 ml 85% H_3PO_4 per gallon).

C Ethyl ether, anhydrous, reagent grade

D Alumina, Fisher A-540

E Celite 545 Johns-Manville

III SPECIAL EQUIPMENT

Sorvall Omni-mixer

IV SAMPLE PREPARATION

The fish should be preserved by freezing as soon as possible after collection. When time permits, it may be skinned and a filet portion selected and cut into 10 or 20 gm portions for extraction. Refreeze these samples.

V EXTRACTION

A Cut the frozen sample of fish tissue quickly into small pieces (roughly 1 cm cubes). Place about 20 gm of the tissue into the Omni-mixer (or Waring Blender, explosion proof) with 175 ml of hexane and about 9-10 gm of Celite 545. Homogenize for about 30 seconds. Remove the stainless steel container and hold under a hot water tap for 1 minute; replace on the mixer and homogenize for 2.5 minutes.

B Decant the supernatant liquid, and mix the tissue with another 175 ml portion of hexane for 3 minutes.

C Decant, combine the extracts, and filter with suction through Whatman #1 paper in a Buchner funnel.

D Evaporate the extract to about 10 ml.

VI HEXANE-ACETONITRILE PARTITION

A Transfer the extract to a 125 ml separatory funnel and bring to a 25 ml volume with hexane. Mark the separatory funnel at the 25 ml mark.

B Add 100 ml of acetonitrile to a 250 ml Erlenmeyer flask (CAUTION: Acetonitrile is extremely toxic; avoid contact with skin). Saturate the acetonitrile with hexane by adding hexane until the solution appears and remains cloudy for a few seconds after swirling (approximately 15 ml of hexane is required).

C To the separatory funnel add 25 ml of acetonitrile saturated with hexane. Gently mix solvents in separatory funnel by rocking back and forth a few times (CAUTION: Release pressure in separatory funnel by opening stopcock).

D Extract pesticides into acetonitrile by gently rocking separatory funnel back and forth for 30 seconds. (NOTE: Severe emulsions may occur. Emulsion will begin to break if allowed to stand for a few minutes. PATIENCE!)

E Drain lower layer (acetonitrile) into Kuderna-Danish evaporator.

F Re-extract with 3 additional 25 ml portions of redistilled acetonitrile saturated with hexane. Collect all acetonitrile extracts in Kuderna-Danish evaporator. Discard hexane fraction.

G Evaporate acetonitrile extract to approximately 10 ml. Evaporate to dryness in a hot water bath under a gentle stream of filtered air. (NOTE: The residue may be a yellow oil if the fish sample has a high fat content. In this case, evaporate until a clear oil is produced.) Take up the residue in 10 ml hexane.

VII COLUMN ADSORPTION CHROMATOGRAPHY

A Prepare slightly deactivated adsorption alumina (1.4%) by adding 0.7 ml distilled water dropwise with a pipette on top of 50 gm of alumina (Fisher A-540) in a small glass jar. Distribute drops over entire surface of alumina. Using a stirring rod mix and break up the lumps formed in the alumina. Cap the jar and tumble gently for 3 minutes to mix the alumina.

B Into a chromatographic column place about 16 cm of deactivated alumina. Tamp to 15 cm with a rubber stopper.

C Place a 50 ml graduate under the column; with another 50 ml graduate add 50 ml hexane (redistilled) to column to prewet it. Just after adding 50 ml of hexane, mark 50 ml level on column with a grease pencil. Adjust elution rate to 3.5 to 4.0 ml per minute (approximately 1 drop per second). When hexane has just reached level of alumina, using a stirring rod transfer the hexane sample extract to the column. Wash sample tube with additional 10 ml of hexane.

D When hexane has just reached level of column, add 50 ml eluting solution (9:1 hexane/ethyl ether). Level of eluting solution in column should correspond to first 50 ml mark. If it does not, remark column at new level. Place a 125 ml Erlenmeyer flask under the column and collect the first 50 ml fraction (mark on a separate sheet of paper the time at which elution begins). Throughout the collection of the fraction, keep the level of the eluting solution at the 50 ml mark on the column; this is important in order that the elution rate of 3.5-4.0 ml/min be maintained. After the first 50 ml fraction has been collected, again mark the time. If elution rate is adjusted properly, collection of the fraction should take 12-14 minutes. Increase or decrease elution rate as required. Begin immediately to collect the second 50 ml fraction. Proceed as in the collection of the first fraction. Collect a total of 5 fractions. Cap with aluminum foil and identify each flask.

VIII ELECTRON CAPTURE GAS CHROMATOGRAPHY

A Prepare a standard curve for the pesticide standards at your work bench by using a gas chromatograph equipped with an electron capture detector.

B Inject 5-10 µl of each fraction (from the alumina column) into the gas chromatograph. Tentatively identify and quantitate the insecticide in each fraction.

C Composite the fractions containing the pesticides and concentrate to 5 ml in a Kuderna-Danish evaporator. Further concentrate to 1-2 ml in a hot water bath with a gentle stream of filtered air. Transfer to a chromatographic storage tube. Further concentrate to 0.2 ml in a hot water bath with a gentle stream of filtered air. When extract reaches 0.2 ml immediately cool in a cold water bath. Rinse walls of tube with 100 µl hexane and concentrate to 0.1 ml in a hot water bath with a gentle stream of filtered air. When extract reaches 0.1 ml immediately cool in a cold water bath and store in the refrigerator until ready for TLC. (NOTE: The heating and cooling steps are essential in preventing losses due to the oily extract creeping up the walls of the tube.)

IX THIN LAYER CHROMATOGRAPHY

A Spotting of Plate

1 Lay a kimwipe open on top of bench. Scrape 1/4 inch area on two sides of plain Al_2O_3. Position plate on kimwipe.

2 Place a kimwipe folded 1/4 size on left hand side of the work area.

3 Place DDE and γ chlordane standard vials (1 μg/μl) and tube containing gamma chlordane fish fraction (in cool water bath) along side of folded kimwipe.

4 Place a template with graduated edge upon the aluminum oxide plate with starting line about one (1) inch from bottom.

5 Mark with a pencil the starting line and 10 cm line on both sides of plate.

6 Insert (5 μl) capillary spotting tube in small beaker of hexane and touch to folded kimwipe until all hexane has been removed from capillary. Repeat this five times.

7 Rinse capillary twice with DDE removing contents each time on kimwipe.

8 At spotting position #1 of template, touch capillary filled with DDE once lightly on plate removing approximately 1/4 of contents of capillary.

9 At position #2 spot two times putting 1/2 the contents of the capillary on the plate. Permit each spot to dry before respotting.

10 Remove contents of the capillary on kimwipe and refill from vial.

11 At position #3 spot the contents of the capillary (5 μl) by lightly touching the capillary to the plate removing about 1/4 of the contents with each spotting. Permit the spots to dry before respotting.

12 Rinse capillary 5 times in hexane.

13 At positions #4 - #6, spot gamma chlordane. Repeat spotting procedure as described in items 8 - 12.

14 Rinse 20 μl capillary in hexane.

15 Insert 20 μl capillary into chromatographic storage tube containing sample. Allow capillary to draw up sample by capillary action only (approximately 0.01 ml). At position #7, spot the contents of the capillary. Refill the capillary and spot again. Continue refilling and spotting until entire sample has been spotted. Always allow spots to dry before respotting.

B Development in Solvent

1 Place plate, coated surface facing solvent saturated paper, in developing tank containing 1% ethyl ether in hexane with marked spotting line down. Develop to 10 cm line.

2 Lift off top of jar and remove plate, lifting plate with finger tips of both hands. Dry in air for 10 minutes.

3 Place plate in hood and spray with Rhodamine B. The spray should be a fine mist giving an even, smooth light pink color to surface of plate.

4 Place plate in dark and observe purple spots with UV light.

5 Measure distance (cm) of spotting line to solvent front. Measure distance of spots from starting line.

6 Calculate R_f of spots which appear in fish sample.

$$R_f = \frac{\text{Distance of spot}}{\text{Distance of solvent front}}$$

C Preparation of Thin-Layered Separated Pesticides for Infrared Spectrophotometry

1 Marking off spot area on plate

a Place template below lower spot line. Mark surface with spatula across plate.

b Move template above these spots and similarly mark surface.

c Move template to a line above the gamma chlordane spots and mark surface. Similarly, mark surface below these spots.

d Turn template perpendicular to selected spot; mark surface across plate.

e Move template to other side of spot and mark surface.

2 Removal of spot from plate

a Insert a tuft of glass wool and position at the tapered end of medicine dropper.

b Mark the medicine dropper to identify the pesticide spot collected.

c Connect vacuum tubing over tapered end of medicine dropper.

d Collect all of adsorbent surface enclosed between boundaries of marked off area by sucking the adsorbent and pesticide by vacuum and depositing in glass wool of medicine dropper.

3 Elution of pesticide

a Mark a 15 ml centrifuge tube.

b Place paper clip over tapered end of medicine dropper approximately 2/3 distance from large end.

c Insert tapered end of dropper into tube supported by paper clip.

d Elute pesticide with 5 ml of 4:1 hexane-ether mixture into 15 ml centrifuge tube.

e Concentrate to 1-2 ml in hot water bath with a gentle stream of filtered air. Transfer to chromatographic storage tube. Further concentrate to 0.2 ml in a hot water bath with a gentle stream of filtered air. When eluate has reached 0.2 ml, immediately cool in a cold water bath. Wash walls of tube with 100 µl hexane. Concentrate to 0.2 ml in a hot water bath with a stream of filtered air. Stopper and store in refrigerator or cool water bath for IR.

X NOTES ON PROCEDURE

A All solvents must be of the highest quality. Even solvents which can be purchased in a redistilled form (e.g. Burdick and Jackson) must be redistilled again in the laboratory.

B The analyst should be careful throughout the procedure to avoid unnecessary loss of pesticide. This can be done by care in evaporation, use of tapered centrifuge tubes for transfer and care in spotting the thin-layer plate.

C Table 1 shows the separation of chlorinated hydrocarbons which should be expected with this column. It may be necessary to increase or decrease the amount of water used for deactivation of the alumina in order to achieve the proper separation.

This outline was revised by W. E. Loy, Chemist, Analytical Services and W. R. Davis, former Chemist, Training Officer, Southeast Water Laboratory, Athens, GA.

Descriptors: Chlorinated Hydrocarbon, Pesticides, Fishkill, Organic Compounds, Organic Pesticides, Pesticide Residues, Pesticide Toxicity

TABLE 1

ORDER OF ELUTION OF STANDARD INSECTICIDES FROM ALUMINA ADSORPTION COLUMN

Insecticide	1	2	3	4	5	6	7	8	9	10	Weight Insecticide Used μg	% Recovery (Avg. of 3 Runs)
DDE	95	5									10	94
Aldrin	93	7									10	97
Heptachlor	70	30									10	96
Chlordane (Tech.)	30	30	40	Trace							20	99
Toxaphene	15	55	30	Trace							50	93
DDT	5	95									20	94
γ Chlordane		2	80	18							10	99
DDD			60	40							10	93
Lindane			35	65							10	40
Endrin				45	55						10	95
Hept. Epoxide				35	50	15					10	95
Dieldrin						20	40	20	15	5	10	96
Methoxychlor						5	30	50	10	5	50	96
Lindane	(Acid alumina)				25	60	15				10	100
Lindane	(Neutral alumina)			- 3	75	20	2				10	91

Eluting Solvent - 9:1 Hexane/Ethyl ether.
Elution Rate - 3.5 to 4.0 ml per minute.

ATOMIC ABSORPTION SPECTROPHOTOMETRY

I INTRODUCTION

Atomic absorption spectroscopy has been well known to physicists and astronomers for more than 100 years. In 1850, Kirchoff took light from the sun and collimated it with a lens through the flame of an ordinary laboratory burner, and then passed the light through a prism which dispersed it into the characteristic visible spectrum with which we are all familiar. He then took a platinum spoon containing a sodium salt and introduced it into the flame. He observed that the yellow light that was present in the spectrum disappeared and in its place appeared the characteristic resonance lines of sodium. Since then astronomers have used the technique to detect and measure the concentration of metals in the vapors of stars. In 1953, Walsh(1) recognized its potential advantage over emission spectroscopy for trace metal analysis. He designed and built an analytically useful atomic absorption instrument. Shortly thereafter the advantages of atomic absorption instrumentation were recognized in the United States.

II THEORY

The basis of the method is the measurement of the light absorbed at the wavelength of a resonance line by the unexcited atoms of the element. Elements not themselves excited to emission by a flame may be determined in a flame by absorption provided that the atomic state is capable of existence.

At the temperature of a normal air-acetylene flame (2100°C) only about one per cent of all atoms is excited to emission in a flame; therefore absorption due to a transition from the ground electronic state to a higher energy level is virtually an absolute measure of the number of atoms in the flame, and the concentration of the element in the sample. Electrons will absorb energy at the same characteristic wavelength at which they emit energy. This is the principle upon which the technique of atomic absorption spectroscopy is based.

The advantages of atomic absorption spectroscopy as compared to emission spectroscopy are: (1) that atomic absorption is independent of the excitation potential of the transition involved and (2) that it is less subject to temperature variation and interference from extraneous radiation and interference from extraneous radiation or energy exchange between atoms.

Atomic absorption analytical apparatus (Figure 1) consists of a suitable source of light emitting the line spectrum of an element, a device for vaporizing the sample, a means of line isolation (monochomator or filter) and photoelectric detecting and measuring equipment.

If the detector is placed to receive only the resonance line of the element from the light source, measurement can be made of the absorption of resonance-line radiation on its passage through the vaporized sample. The magnitude of this absorption gives a measure of the concentration of free ground-state atoms of the element in the vapor and when referred to a calibration curve, provides a means of determining the concentration of the element in the sample.

III INSTRUMENTATION

The general arrangement of an absorption flame photometer is no different from an emission flame photometer except for the addition of a light source. An aerosol is introduced into a flame which is placed on the optical axis between the entrance slit of the monochromator and the monochromatic light source. Energy of the wavelength absorbed by the sample is provided by a source lamp whose emitting cathode is made of that element. This energy is passed through the flame and then through a dispersing device. A detector measures the absorbed and unabsorbed exciting radiation.

A Light Source

For the more volatile elements such as the alkali metals, mercury and thallium,

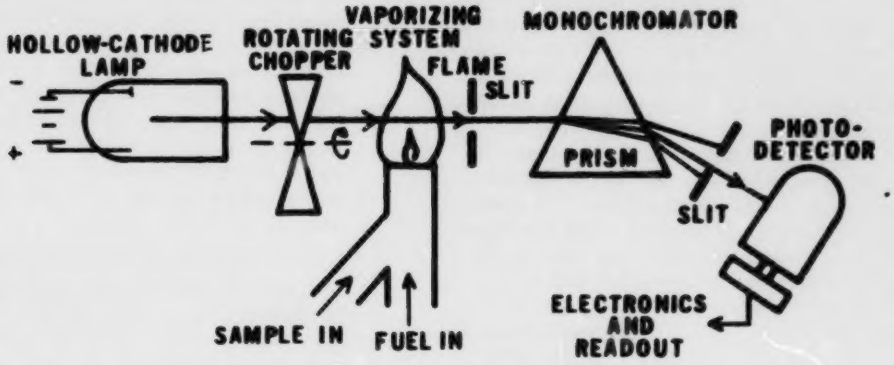

HOLLOW-CATHODE LAMP
ROTATING CHOPPER
VAPORIZING SYSTEM
FLAME
SLIT
MONOCHROMATOR
PRISM
PHOTO-DETECTOR
SLIT
SAMPLE IN
FUEL IN
ELECTRONICS AND READOUT

FIGURE I

the most convenient source is the spectral vapor lamp, which consists of a closed glass or silica tube, into which are sealed oxide-coated electrodes, containing one of the rare gases and some of the appropriate metals. For line sources of the less-volatile elements, hollow-cathode discharge tubes have been found the most satisfactory. These consist of an anode and hallow cylindrical cathode (either composed of or lined with the appropriate metal) mounted in a sealed glass tube containing one of the rare gases (helium or argon).

Lamps are operated at low currents to improve linearity of response and maintain narrow emission lines.

B Vaporization of Sample

1 Atomic-absorption methods have been applied almost exclusively to the analysis of solutions and for this purpose flames, fed with a fine spray of the sample solution, similar to those employed in flame photometry are used.

The burner has two principal functions to perform:

a It must introduce the sample into the flame.

b It must reduce the metal to the atomic state.

2 Burners can be classified as:

a Total-consumption

Those which introduce the sample spray directly into the flame. (Figure 2)

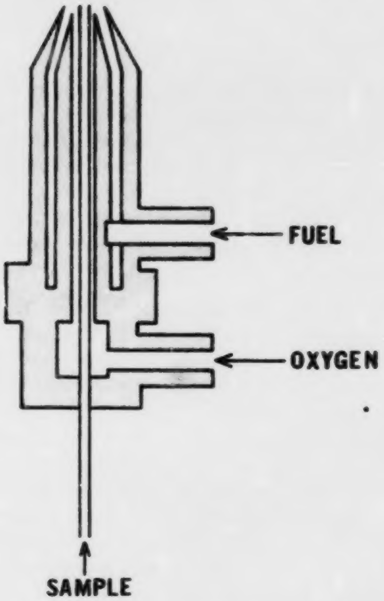

FUEL
OXYGEN
SAMPLE

FIGURE 2

b Premix

Those which introduce the spray into a condensing chamber for removing large droplets. (Figure 3)

3 Flame shape is important. The flame should have a long path length (but a narrow width, such as a fishtail flame) so that the source traverses an increased number of atoms capable of contributing to the absorption signal.

4 The effective length of the flame may be increased by multiple passages through the flame with a reflecting mirror system, or by alignment of several burners in series.

5 The flame temperature need only be high enough to dissociate molecular compounds into the free metal atoms.

Typical flame temperatures are shown in Table I.

Table I

Fuel-Oxidant	Approximate Temp., °C
Nitrous Oxide - acetylene	3000
Hydrogen - air	2100
Hydrogen - oxygen	2700 - 2800
Acetylene - oxygen	3100
Acetylene - air	2000 - 2200
Propane - oxygen	2700 - 2800
Illuminating gas - oxygen	2800
Cyanogen - oxygen	4800

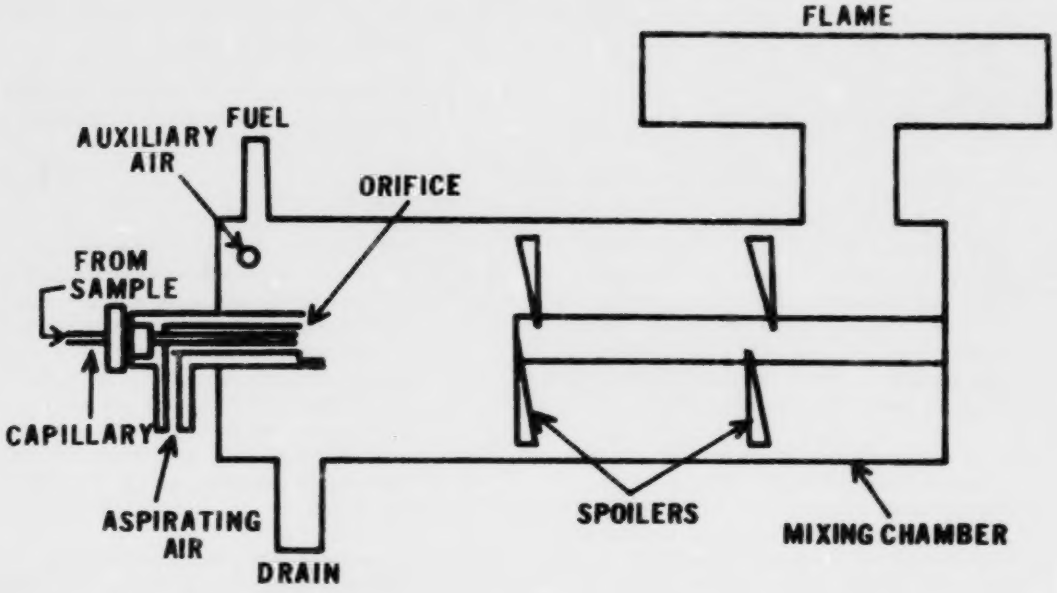


Figure 3

C Line Isolation

1 The use of a line spectrum of the element being determined, rather than a continuous spectrum, makes possible the use of monochromators of low resolving power or even filters. When a spectral lamp is used as a light source, it is only necessary to isolate the resonance line from neighboring lines of the light source or vaporized sample. The resolution of the method is implicit in the width of the emission and absorption lines.

2 To realize the full potentialities of the method, the strongest absorption line must be used. For elements with simple spectra, the resonance line arising from the lowest excited state is usually the line exhibiting strongest absorption.

3 Calibration curves depart from linearity at much lower concentrations in absorption work as compared with emission work. Curvature results partly from increased pressure broadening as the concentration of salt rises, but also depends on source characteristics, particularly self-absorption, and on the nature and homogeneity of the flame.

D Detection

1 Photo-electric detectors used in atomic absorption analysis need be no more sensitive than those used in emission analysis, since in the atomic absorption method, concentration of an element is determined by measuring the reduction in intensity of the resonance line emitted from a source of high intensity.

2 Single or double-beam circuits may be adopted for work with a single beam instrument, results are directly dependent upon source and detector stability. Both must be powered by separate power supplies. In a double-beam system small variations in the source signal are compensated automatically.

IV EVALUATION

A Sensitivity

1 For an air-acetylene flame of length 2 or 3 cm the lower limits of detection of elements having low resonance-line excitation potential (eg Na-K) are approximately equal in a single-beam atomic absorption and emission methods.

2 For elements having highly reversed resonance lines or resonance lines of high excitation potential, the atomic-absorption method has decided advantages over emission methods. Examples of elements in these categories are Zn, Mg, Fe and Mn.

3 A disadvantage of the atomic-absorption method, when compared with flame emission, is the lack of a quick and simple method of varying sensitivity to deal with solutions of widely varying element concentrations. The sensitivity of an atomic-absorption instrument is determined almost entirely by flame characteristics, notably length of light path through the flame.

4 A comparison of sensitivity obtained by emission and adsorption techniques is given in Table II.

B Precision

1 Precision of a single-beam atomic absorption instrument is primarily a function of the stability of light output from the spectral lamp. This in turn is dependent on the stability of the main supply and inherent stability of the lamp. The largest fluctuations are only ± 2 percent for the hollow cathode tube and sodium spectral vapor lamp. A double-beam instrument significantly reduces this error.

2 In common with flame-emission methods, atomic absorption is subject to "noise" from the flame and the detector. Changes in absorption caused by fluctuations in

Element	Sensitivity mg/l	
	Flame	A. A
Aluminum	2	0.5
Antimony		0.2
Arsenic		1.0
Barium	0.3	1.0
Beryllium	25	0.05
Bismuth		0.2
Cadmium	2	0.01
Calcium	0.003	0.01
Cesium		0.05
Chromium	0.1	0.01
Cobalt		0.15
Copper	0.01	0.005
Gallium		1.0
Gold		0.1
Indium		0.5
Iron	0.2	0.05
Lead	2	0.15
Lithium	0.002	0.005
Magnesium	0.1	0.003
Manganese	0.01	0.01
Mercury	10	0.5
Molybdenum		0.2
Nickel		0.05
Palladium		1.0
Platinum		0.5
Potassium	0.001	0.005
Rhodium		0.3
Rubidium		0.02
Selenium		1.0
Silver	0.05	0.02
Sodium	0.002	0.005
Strontium	0.01	0.02
Tellurium		0.5
Titanium		1.0
Thallium		0.2
Tin		2.0
Vanadium		0.5
Zinc	200	0.005

Table II

flame temperature are much less than those in emission because the strength of the absorption line is principally dependent on Doppler width whereas the intensity of emission from the flame is much more sensitive to temperature.

C Accuracy

This is shown by the types of interference found in flame emission and atomic absorption spectroscopy. There are three types:

1 Physical

Collision of atoms and electrons or atoms and molecules will transfer energy thus causing an enhancement or depression of analysis-line emission. This has a large effect on flame emission analysis but has only a negligible effect on atomic absorption.

2 Radiative

Light from elements other than the one being measured pass the line isolating device (monochromator or filter). This occurs in flame emission work, for example, the interference of calcium and magnesium in sodium determinations. This interference is also encountered in atomic absorption using a D. C. system but is very small because of the large signal from the hollow-cathode tube. Radiative interference is eliminated in an A. C. system.

3 Chemical

Emission from an element in the flame is depressed by the formation of compounds, which are not dissociated at flame temperatures. This also affects absorption because the formation of temperature - stable compounds in the flame causes proportionate reduction in the population of ground-state and excited atoms.

Investigations to date suggest chemical interference is confined, almost entirely, to the alkaline-earth elements and that calcium absorption is more subject to this interference than is magnesium absorption.

Typical calibration curves are shown in Figure 5.

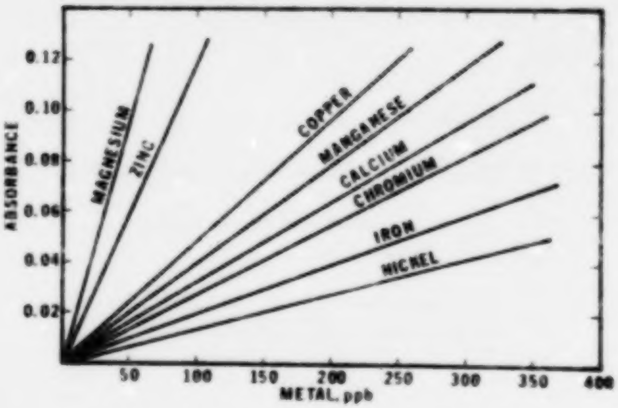


Figure 5

V REMOVAL OF INTERFERENCES AND CONCENTRATION OF SAMPLE

A Removal of Interferences

1 The methods for overcoming these interferences in atomic absorption are similar to those used in flame emission, namely, either separation of interfering ions or suppression of the interference by addition in excess of a substance that will prevent formation of compounds between interfering ions and the element being determined.

B Concentration of Sample

1 Organic separations can be used to concentrate a sample. Interferences are removed, as seen above, and also the organic solvent enhances the absorption.

2 Ion exchange has also been used successfully for concentrating samples for atomic absorption.

VI CONCLUSIONS

Atomic-absorption methods are as good as or better than emission methods, for elements to which they can both be applied, in sensitivity, precision and accuracy. They can be applied to a far wider range of elements than can emission analysis. The additional cost of hollow cathode discharge tubes is compensated by the greater range of analyses and greater reliability of results.

VII INSTRUMENTS AVAILABLE

A Perkin Elmer

1 Model 303 - double beam, AC -$5,920.00

2 Model 290 - single beam, AC -$2,900.00

B Beckman attachments for existing spectrophotometers.

1 Use with model D.U. and D.U. -2- single beam, DC - $2,135.00

2 Use with model D.B. - single beam, AC - $2,495.00

C Jarrell-Ash

1 Dual atomic absorption flame spectrometer - single beam, AC - $5,800.00

D E.E.L.

1 Atomic absorption spectrophotometer - single beam, AC $2,850.00.

ACKNOWLEDGEMENT:

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This outline was prepared by Paul F. Hallbach, Chemist, National Training Center, MDS. OWP, EPA, Cincinnati, OH 45268

Descriptors: Absorption, Chemical Analysis, Instrumentation, Metals Analysis, Spectrophotometry, Water Analysis

METALS IN NATURAL AND TREATED WATERS IN THE ABSENCE OF GROSS POLLUTION BY ATOMIC ABSORPTION SPECTROPHOTOMETRY

Most metals can be determined by atomic absorption with an air-acetylene flame (see JAWWA, 60:739, 1968). A few, however, require a higher temperature flame to dissociate the molecules. Metals which can be determined by use of the higher temperature nitrous oxide-acetylene burner include aluminum, beryllium and barium. While barium can be determined by aspiration of the aqueous solution directly into the flame, the method is not sensitive enough to determine aluminum and beryllium at the concentrations of interest to water plant operators, unless concentrated by complexing with a chelating compound and extracting into an organic solvent.

I GENERAL DISCUSSION

A Principle

Atomic Absorption resembles emission flame photometry in that a sample is aspirated into a flame and atomized. The major difference rests in the fact that flame photometry measures the amount of light emitted, whereas in atomic absorption spectroscopy a light beam is directed through the flame, into a monochromator, and onto a detector which measures the amount of light absorbed. Absorption is more sensitive because it depends upon the presence of free unexcited atoms and in the usual flames the ratio of unexcited to excited atoms at a given moment is very high. Since each metallic element has its own characteristic absorption wavelength, a source lamp composed of that element is employed for each element and hence the method is relatively free of spectral or radiation interferences. The amount absorbed in the flame is proportional to the concentration of the element in the sample.

B Interference

The most troublesome type of interference is usually termed "chemical" and results from the lack of absorption from atoms bound in molecular combination in the flame. This phenomenon can occur when the flame is not sufficiently hot to dissociate the molecule or because the dissociated atom is immediately oxidized to a compound that will not dissociate further at the temperature of the flame. Barium fits into the former and aluminum and beryllium into the latter category and as a result, the nitrous oxide-acetylene flame is recommended because of its higher temperature. In addition, barium may undergo ionization in the flame, and the ground state (potentially absorbing) population is thereby reduced. This situation can be overcome by the addition of an excess of a cation having a similar or lower ionization potential.

II APPARATUS

A Atomic absorption spectrophotometer.

B Burner

Nitrous oxide burner head with 2 inch slot (reduces overheating frequently encountered with 3-inch slot). When using a nitrous oxide acetylene flame always remember to clean the burner slot frequently using a razor blade or equivalent prior to ignition to dislodge the carbon crust which forms along the slit surface approximately every 20 minutes.

C Separatory funnels

250 ml, preferably with teflon stopcocks, for extractions with organic solvents.

D Glassware

All glassware, including sample bottles, should be rinsed with 1 + 1 HNO_3 followed by deionized distilled water to avoid contamination errors.

III REAGENTS

A Deionized distilled water

Prepare by passing distilled water through a mixed bed of ion-exchange resins. Use deionized distilled water for the preparation of all reagents, calibration standards, and as dilution water.

B Nitric acid, conc.

C Sodium chloride solution

Dissolve 250 grams of reagent grade sodium chloride in distilled water and make up to 1000 ml.

D Stock barium solution

Dissolve 1.7787 grams barium chloride ($BaCl_2 \cdot 2H_2O$) in 1000 ml distilled water to obtain a solution containing 1.0 mg/ml barium.

E Stock beryllium solution

Dissolve 20.7574 grams of beryllium nitrate, $Be(NO_3)_2 \cdot 3H_2O$, in 1000 ml distilled water to obtain a solution containing 1.0 mg/ml beryllium.

F Stock aluminum solution

Dissolve 8.792 g of potassium aluminum sulfate, $K_2Al_2(SO_4)_2 \cdot 24H_2O$ in distilled water and dilute to 1 liter in a volumetric flask to obtain a solution containing 0.5 mg/ml of aluminum.

G Standard solutions

In general, prepare a series of standard solutions containing 0.005 to 1.0 mg/l metal by appropriate dilution of the stock solution.

H Fuel and oxidant

Use nitrous oxide-acetylene flame for all the metals in Table I.

I Special reagents for determining low concentrations of aluminum and beryllium

1 8-Hydroxy-quinoline

Dissolve 20 grams in 1 N acetic acid (60 ml glacial acetic acid per liter) and make up to a volume of 1 liter with the 1 N acid.

2 Buffer

Dissolve 200 grams of ammonium acetate and 70 ml of concentrated ammonium hydroxide in distilled water and make up to a total volume of 1 liter.

3 Chloroform

IV PROCEDURE

A Pretreatment of sample

Acidify all water samples with nitric acid (1 ml concentrated nitric acid per 100 ml sample) and autoclave at 250°F for one hour to solubilize particulate matter, and readjust volume, if necessary.

B Precaution

Install the spectrophotometer on a level bench or table with the burner under a vent.

C Instrument operation

Because the differences between the makes and models of satisfactory atomic absorption spectrophotometers render impossible the formulation of detailed instructions applicable to every instrument, follow the manufacturer's operating instructions. As a rule, choose the correct hollow cathode lamp, install and align in the instrument; position the monochromator at the correct wavelength (Table I); select the proper monochromator slit width; set the light source current according to the manufacturer's recommendation; light the flame and regulate the flow of fuel and oxidant; adjust the burner for maximum percent absorption and stability; and balance the photometer. Then run the standards which are needed for the working curves and plot the concentrations of the standards against absorbance. If necessary on some instruments, convert percent absorption to absorbance. (The calibration curves are nearly linear for most elements.) Next run the samples and read the concentration of each sample from the calibration curve. For best results run standards each time a sample or series of samples are run.

D Special procedure for barium

To overcome ionization interference in the flame, add 2 ml sodium chloride solution to 100 ml sample before aspiration. Prepare standards similarly to contain 2000 mg/l sodium.

TABLE I

Metal	Wavelength mμ	Sensitivity (mg/l per 1%)[a]
Aluminum	309.3	1.0[b]
Barium	553.6	0.2
Beryllium	234.8	0.1[c]

[a] mg/l of metal for 1% absorption in an aqueous solution.

[b] 0.05 if extracted.

[c] 0.005 if extracted.

E Special procedural steps for low concentrations of aluminum and beryllium

To 100 ml of sample add 2 ml of 8-hydroxy quinoline solution and 3 ml of buffer solution. Adjust the mixture, if necessary to a pH of 8.0 ± 0.5 and then extract with 5 ml of chloroform. Repeat the extraction with a second 5 ml of chloroform and combine the two extracts. Determine the amount of metal in the organic phase by comparison with standards which have been similarly extracted.

F Aspiration of an organic solvent into the flame

When working with organic solvents, reduce the fuel-to-air ratio because the burning of the organic solvent contributes to the fuel supply and in addition to lifting the flame off the burner may produce an undesirable luminescent flame. In setting the fuel-to-air ratio of the gas mixture at the burner, start with the settings recommended by the manufacturer for the analysis and gradually reduce the fuel flow (while the solvent is being aspirated) until the flame is similar to the flame before aspiration of the organic solvent. With the nitrous oxide burner approximately double the flow of acetylene is required over that normally used for the air-acetylene burner and the acetylene should be adjusted until the flame is a rose-red.

V CALCULATION

A For aqueous sample refer directly to the calibration curve

$$\text{mg/l metal} = (\text{mg/l in aliquot}) \times D$$

where

$$D = \text{dilution ratio} = \frac{\text{ml sample} + \text{ml water} + 1\ (\text{ml acid})}{\text{ml sample}}$$

B For extracted sample refer to the extracted standard calibration curve.

$$\text{mg/l metal} = (\text{mg/l in aliquot})$$

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This outline was prepared by Earl F. McFarren, Chief, Analytical Quality Control, Bureau of Water Hygiene, EPA, Cincinnati, OH 45268

Descriptors: Absorption, Chemical Analysis, Instrumentation, Laboratory Tests, Metals Analysis, Spectrophotometry, Water Analysis

PRINCIPLES OF EMISSION SPECTROSCOPY

I INTRODUCTION

The term "spectroscopy", in its broadest sense, refers to the study of the radiations of the electromagnetic spectrum. As no single instrument exists which will separate radiation from all parts of the spectrum, it is divided into regions which are related to the different types of instruments capable of producing or measuring waves of various lengths. A diagram of the spectral distribution of energy is shown in Figure 1.

Emission spectroscopy is concerned with that region wherein radiation can be sorted out into a spectrum by means of prisms or gratings, and includes the near infrared, the visible and the ultraviolet.

Emission spectroscopy has become an indispensable part of the modern chemist's analytical methods. With it he can analyze a wide variety of substances, both qualitatively and quantitatively, for trace elements regardless of valence states.

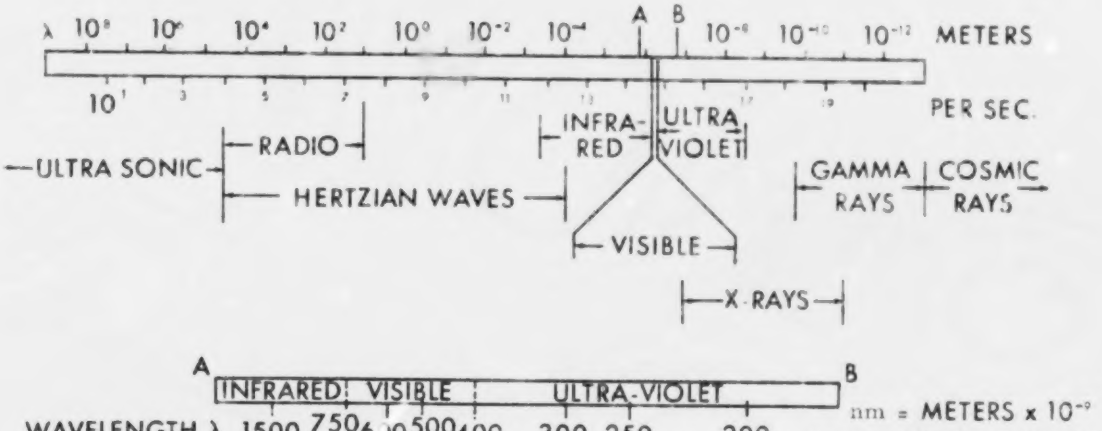
II THEORY

A **Origin of Spectra** - Excited atoms and atomic ions emit light of definite wavelengths. The excitation of multiple elements in a sample results in the simultaneous production of the spectra of all of these elements.

During excitation by a thermal or electrical source, the outer orbital electrons of an element absorb energy and rise to higher energy levels. These electrons then return spontaneously to their normal or ground state by a single jump or by a series of jumps. The energy emitted with each jump produces a spectral line of characteristic wave length and frequency for the particular chemical element. The combination of lines produced by the excited atoms of the element thus provides the emission spectrum of that element.

FIGURE 1

SPECTRAL DISTRIBUTION OF ENERGY


λ
10⁸
10⁶
10⁴
10²
10⁰
10⁻²
10⁻⁴
A
B
10⁻⁸
10⁻¹⁰
10⁻¹²
METERS
10¹
3
5
7
9
11
13
17
19
PER SEC.
RADIO
ULTRA SONIC
HERTZIAN WAVES
INFRA-RED
ULTRA VIOLET
GAMMA RAYS
COSMIC RAYS
VISIBLE
X-RAYS
A
B
INFRARED
VISIBLE
ULTRA-VIOLET
WAVELENGTH λ
1500
750
600
500
400
300
250
200
nm = METERS x 10⁻⁹
1 nm = 10 ANGSTROMS

REGION INCLUDED IN EMISSION SPECTROSCOPY

B Types of Spectra - There are three types of emission spectra.

1 The line spectra produced by highly excited atoms or atomic ions.

2 Band spectra which originate with highly excited molecules.

3 Continuous spectra which result when light is emitted by incandescent solids.

III INSTRUMENTATION

A The emission spectrograph consists of essentially four distinct functional parts: the excitation source, the slit, the optical system, and the recording system. A diagram of a Littrow type spectrograph is shown in Figure 2.

1 The production of line spectra for the detection or the determination of the elemental constituents in a sample requires an excitation source, such as an arc, a spark, or a flame.

2 The slit of a spectrograph permits only a narrow beam of light of mixed wave lengths to enter the instrument.

3 The optical system consists of a series of lenses and either a prism or grating to separate light rays of different wave lengths into the spectrum.

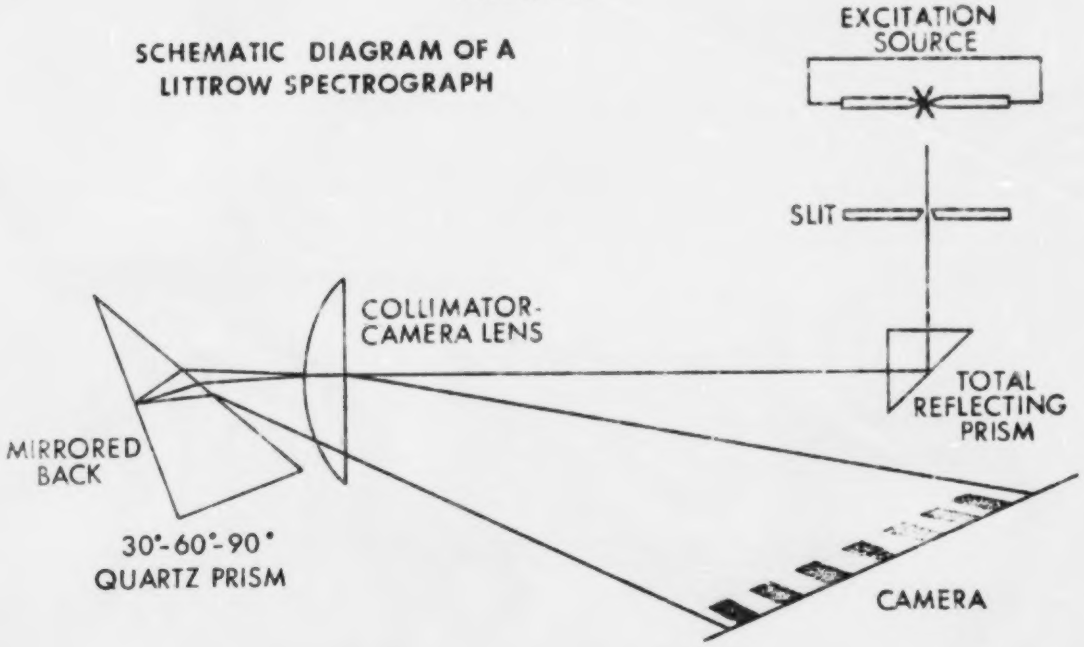
4 The recording system can be

a An eye piece such as used in a spectroscope.

b A photographic plate such as used in a spectrograph.

FIGURE 2

SCHEMATIC DIAGRAM OF A LITTROW SPECTROGRAPH



c A photoelectric cell like that used in direct reading spectrographs.

IV SELECTION OF EQUIPMENT AND COST CONSIDERATIONS

A A decision concerning the advisability of installing a laboratory for spectrochemical analysis, whether in an industrial plant, research laboratory or health department, might rest upon four main considerations.

1. Whether the analytical problems encountered have a satisfactory solution other than spectroscopy.
2. The time urgency of the work.
3. The expected volume and diversity.
4. The cost of the initial investment, together with operating expenses.

V PROBLEMS AND TECHNIQUES ASSOCIATED WITH EMISSION SPECTROSCOPY

A After the equipment has been set up properly and is ready for operating, there are many parameters to be determined before the first samples are analyzed.

1 Source - arc or spark

There are several types of excitation sources available, as shown in Table 1. Generally, the type of sample and the precision and accuracy required for the analysis will govern which excitation method will be employed.

2 Electrode

The spectrographic electrode plays an important part in the analysis. Some important features are density, shape and purity.

Table 1. EXCITATION SOURCES

TYPE	PRECISION	SENSITIVITY	RANGE	USE
D.C. ARC	Fair	Excellent	0.00001 - 1.0%	Basic sources for general qualitative analysis - soils, ores, oxides, slags, ashes, etc. Highest sensitivity of detection for trace elements.
HIGH VOLTAGE A.C. SPARK	Excellent	Fair	0.01 - 30.0%	Most stable. Use for alloying constituents in metals. Solution techniques.
LOW VOLTAGE A.C. ARC	Good	Good	0.001 - 1.0%	Quantitative determination of residual impurities for low alloying constituents in metals.
SPARK IGNITED UNI - ARC	Good	Good	0.001 - 1.0%	Combines precision of the spark and the sensitivity of the D.C. arc.
HIGH VOLTAGE A.C. ARC	Fair	Excellent	0.00001 - 1.0%	Steadier than D.C. arc - Conductors and nonrefractory materials.

a Density - The current and temperature obtainable are dependent on the density of the graphite.

b Shape - The nature of the sample being analyzed will usually determine the shape and type of electrode.

c Purity - Trace analysis requires that the electrodes be of very high purity.

3 Exposure conditions

Current, voltage, exposure time, etc., play an important part.

4 Photographic plates

The photographic emulsion most generally used for quantitative analysis is the S.A.#1. This emulsion responds to the spectral range from 2400 - 4400 A. It has high contrast, low background density, and low granularity.

The S.A.#2 emulsion is also desirable for trace analysis when lower contrast, higher speed, and wide latitude are needed. S.A.#2 also covers the range from 2400 - 4400 A. Other photographic emulsions covering the wavelength range from 2400 - 12,000 A are available, each having specific features. The processing of these photographic plates is extremely important and the directions of the manufacturer should be followed closely.

B Qualitative Analysis

In spectroscopy, qualitative analysis is a relatively simple process, although it can be more time consuming than a quantitative determination, especially if a complete qualitative analysis is required. The presence or absence of more than sixty of the chemical elements can be determined readily by a simple inspection of the resulting pattern of spectral lines. All elements give specific lines when sufficiently excited, which is the basis of qualitative spectrochemical analysis.

C Quantitative Analysis

Quantitative spectrochemical analysis is based on the fact that the amount of light emitted by an element present at very low concentration is directly proportional to the number of its excited atoms present, if all other factors are kept constant. The intensity of the spectral line of the analysis element (or degree of blackening of the photographed image of the line) in an unknown sample is compared to the intensity of the corresponding line in a standard sample to provide an estimate of the concentration of the element producing that line.

D Preparation of Standards

1 Since the major constituents of a sample are important factors affecting burning qualities of the arc and intensity of resulting spectral lines, it is important that the composition of the standards approximate the samples as closely as possible. This is accomplished by preparing a synthetic matrix to approximate the average composition of the samples minus the analysis element or elements.

2 Increasing concentrations of the analysis elements are added to the synthetic matrix and are sparked under the identical conditions used for the unknown samples for calibration purposes.

3 It should be emphasized that in quantitative spectrographic analysis, the best possible standards must always be prepared. A good spectrographer is only as good as his analyses, and his analyses are only as good as his standards. Spectrographic standard solutions are prepared from Reagent Grade chemicals. Oxides are prepared where possible; however, nitrates or chlorides will usually suffice.

Occasionally, it is necessary to use special spectroscopically pure grades of certain metals. Nitric and hydrochloric acids should be redistilled; double distilled water is also suggested where possible. Borosilicate glassware, cleaned with both chromic and nitric acids, is used throughout.

4 Internal standards

In quantitative analysis, the errors caused by temperature fluctuations and wandering of the arc are minimized by the use of the internal standard technique. This consists of comparing the intensity of a suitable line of analysis element in a standard and in an unknown sample to a certain selected line of another element whose concentration is fixed in all samples.

Since both the unknown and the internal standards are parts of the sampe sample, variations in time exposure, plate characteristics, and developing conditions will not affect the relative density of the two lines which are equal in intensity in the light source.

Working curves are established by plotting the intensity ratio of the analysis line to the internal standared line vs. the concentration of the analysis element.

E Accuracy

The average error in quantitative emission spectroscopy, using the photographic process, is generally between ±5 - 10%. In the direct reading process, however, where photomultiplier tubes are substituted for the photographic plate, an average error of ±2% is possible. This accuracy is more than satisfactory, considering that determinations of trace elements are being made in the parts per billion range.

VI APPLICATION OF EMISSION SPECTROSCOPY TO WATER ANALYSES

A Trace elements in water originate from a variety of sources, but can be classified into three principal groups.

1 Elements contributed by soluble materials chemically weathered from rocks and soil.

2 Elements that are selectively concentrated by vegetation and find their way to surface waters following decay and run-off.

3 Industrial sources, especially those devoted to mining, alloying, and cleaning and plating of metals.

Such sources may contribute significant quantities of trace elements to surface waters in areas both populated and unpopulated. The detection and measurement of these trace elements are difficult with conventional analytical procedures because they are not adapted to large numbers of samples, and in some cases they are not sensitive enough. The use of spectrographic procedures for routine monitoring of raw waters, however, is admirably suited to the purpose since a large number of elements may be determined simultaneously with a high degree of accuracy.

B Trace elements, whether in finished or raw waters, are generally present in concentrations too low to measure directly with the spectrograph. Therefore, a means of concentration is necessary before the examination can be completed. This can be accomplished in several ways (e. g., evaporation, precipitation, ion exchange, etc.)

1 The method employed by the Environmental Protection Agency, Research and Monitoring Office, consists of evaporating a filtered volume of sample at low heat on a hot plate.

A volume of sample is chosen containing 100 mg of dissolved solids and evaporated to a volume of 5.0 ml or 20,000 mg of solids/l. (The dissolved solids consist for the most part of salts of sodium, potassium, calcium and magnesium; these are the materials for which corrections must be made later.) All samples, therefore, had the same final salt concentration, but the procedure produces varying levels of sensitivity for different sources because of the dissimilar initial volumes. Thus, the limit of detection for trace elements in the Columbia River is lower than for the Missouri River because the low dissolved solids in Columbia River waters permit a larger volume of sample to be evaporated.

a This might be explained best by examining the detection limits shown in Table 2. For convenience, we have arbitrarily chosen 400 mg/l as an average TDS content of surface water, and the detection limits as shown are based on this figure. For waters of only 200 mg TDS/l, the limit would be halved; conversely, if the TDS is 800 mg/l, the limit is doubled.

Table 2. DETECTION LIMITS*, μg/l

Zinc	20	Copper	10
Cadmium	20	Silver	2
Arsenic	100	Nickel	20
Boron	10	Cobalt	20
Iron	10	Lead	40
Molybdenum	40	Chromium	10
Manganese	10	Vanadium	40
Aluminum	40	Barium	2
Strontium	4	Beryllium	0.1

*In water having 400 mg TDS/l.

b The term "detection limit" is used to indicate the lowest concentration of the material that can be distinguished from background noise or interference. The limits, as shown in the table, are based on practical observations and are not mathematically contrived figures based on signal to noise ratios or other formulas.

c When an element has not been positively identified in a sample, the detection limit, preceded by a "less than" sign (<), is used to report the analysis. It should be emphasized that the failure to detect a particular element does not mean that the element is absent; it may be present in the original sample but in a concentration below the stated detection limit. The complete spectrographic procedure has been published previously. Table 3 lists the results from analyses of over 1500 samples. The frequency of detection, the range, and the mean concentrations are given.

2 Accuracy

Recoveries were run with a composite of samples from the Missouri River. The composite was analyzed to determine what trace elements, if any, were present and at what concentrations. To a series of aliquots of this composite were added increasing concentrations of the analysis elements. The samples were acidified, evaporated on a hot plate, transferred to cylinders, and finally sparked under identical conditions. Scaler counts were converted to concentrations by using the data obtained from the synthetic matrix and the percent recoveries were calculated. A statistical treatment of this data showed a range of 80% to 113% at the 90% confidence level.

TABLE 3. SUMMARY OF TRACE ELEMENTS IN WATERS OF THE UNITED STATES*

ELEMENT	No. of Pos. Occurrences	Frequency Of Detection, %	Observed Values, µg/l		
			Min.	Max.	Mean
Zinc	1207	76.5	2	1183	64
Cadmium	40	2.5	1	120	9.5
Arsenic	87	5.5	5	336	64
Boron	1546	98.0	1	5000	101
Phosphorus	747	47.4	2	5040	120
Iron	1192	75.6	1	4600	52
Molybdenum	516	32.7	2	1500	68
Manganese	810	51.4	0.3	3230	58
Aluminum**	456	31.2	1	2760	74
Beryllium	85	5.4	0.01	1.22	0.19
Copper	1173	74.4	1	280	15
Silver	104	6.6	0.1	38	2.6
Nickel	256	16.2	1	130	19
Cobalt	44	2.8	1	48	17
Lead	305	19.3	2	140	23
Chromium	386	24.5	1	112	9.7
Vanadium	54	3.4	2	300	40
Barium	1568	99.4	2	340	43
Strontium	1571	99.6	3	5000	217

* 1577 Samples (October 1, 1962 - September 30, 1967)
** 1464 Aluminum Samples

TABLE 4. RECOVERIES OF TRACE ELEMENTS ADDED TO RIVER WATER

Element	Percent Recovery Low	High	Average
Zn	86.	107.	94.1
Cd	100.	106.	103.5
As	80.	100.	93.5
B	82.	97.	88.9
P	70.	110.	91.8
Fe	85.	108.	94.1
Mo	75.	100.	83.7
Mn	94.	105.	99.1
Al	96.	125.	106.7
Be	101.	117.	109.2
Cu	96.	113.	100.8
Ag[a]			
Ni	90.	118.	103.0
Co	95.	103.	99.3
Pb	60.	93.	83.6
Cr	60.	105.	86.3
V[a]	100.	110.	104.0
Ba	70.	97.	81.7
Sr	81.	112.	95.1

a Silver precipitated and was not included.

Table 4 lists the range of these per cent recoveries along with the average recovery for each element.

C Analytical Reference Service Results

A sample containing 8 trace metals at known concentrations was analyzed by 66 participating agencies using a number of analytical methods. The mean results of all 66 laboratories, in addition to those laboratories employing spectrographic procedures, are shown in Table 5. It is apparent from this summary that the spectrograph can be used to splendid advantage on water samples.

SUMMARY

The theory and instrumentation of emission spectroscopy are presented. Consideration is given to the problems and techniques associated with trace element analysis in water using the Emission Spectrograph.

For the present time, atomic absorption is the method of choice for metals analyses in the EPA Methods Manual. (8)

When broad spectrum analyses are required, the Emission Spectrograph may be used with comparable accuracy and precision.

Table 5. SUMMARY OF RESULTS OBTAINED BY SPECTROGRAPHIC PROCEDURES ON ARS SAMPLE: WATER METALS, NO. 2.

Element	Amount added mg/l	Mean of 66 Labs	AMOUNT RECOVERED mg/l		
			Lab #71126	NWQN Lab	Lab #1615
Al	1.80	2.21	1.80	2.30	3.10
Cr	0.18	0.14	0.18	0.13	0.17
Cu	0.42	0.43	0.29	0.38	0.41
Fe	0.62 **	0.44	0.46	0.40	0.75
Mn	0.25	0.28	0.25	0.23	0.38
Cd	0.24	0.26	0.25 *	0.30	0.50
Zn	0.90	0.94	0.68	0.94	0.88
Pb	0.18	0.20	0.27	0.19	--

** Iron value was adjusted to mean value as suggested on Page 47 of ARS report.

* This result was erroneously included under Lab #7112A in the ARS report.

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This outline was prepared by J.F. Kopp, Chief, Metals Analyses Unit, Analytical Quality Control Laboratory, NERC, EPA, Cincinnati, OH 45268.

Descriptors: Heavy Metals, Instrumentation, Metals, Spectroscopy, Water Analysis

APPLICATION OF EMMISSION SPECTROSCOPY IN WATER ANALYSIS

I DISCUSSION OF LABORATORY RESULTS

II ANALYTICAL PROCEDURES

- A Sample Preparation
- B Exposure Conditions
- C Standard Curves
- D Photographic Vs. Direct-readers
- E Detection Limits

III CHEMICAL SEPARATIONS

- A Ion Exchange
- B Precipitation
- C Chelation and Extraction

IV INTERFERENCES

- A Effect of Major Constituents
- B Spectral Interference
- C Internal Standards

V EMISSION SPECTROSCOPY VS ABSORPTION SPECTROSCOPY

- A Arc - Spark Vs. Flame
- B Flame Vs. Absorption

VI FUTURE OF EMISSION SPECTROSCOPY

- A **Plasma** Arcs
- B Use of Computers

This outline was prepared by J. F. Kopp, Chief, Metals Analyses Unit, Analytical Quality Control Laboratory, FWQA.

Descriptors: Heavy Metals, Instrumentation, Metals, Spectroscopy, Water Analysis

APPLICATIONS OF INTEGRATED MULTIPARAMETER WATER QUALITY DATA ACQUISITION SYSTEMS IN POLLUTION SURVEILLANCE

I BASIC OBJECTIVES OF POLLUTION SURVEILLANCE

The fundamental objective in pollution surveillance is to accumulate sufficient knowledge of stream quality and employ this information to maximize the quality within the framework of established standards and organizations.

A Base-line Data

The quality of a particular stream must be established to determine its relationship with the overall quality imposed upon a particular basin. This is determined by:

1 Grab sample

2 Automatic water quality data acquisition.

B Data Accrued During Changes in Pollution Causes

Automatic surveillance provides transitory data during changes in pollution abatement at the source:

1 Acid mine drainage

2 Dredging operation

3 Improvements in waste treatment.

C Continuous Monitoring

Continuous surveillance of streams and rivers provides research and enforcement data and establishes the effectiveness of the pollution control program.

D Stream Management

Strategically located water quality instrumentation systems with appropriate communication linkages can be employed in the control of reservoir discharge (low flow augmentation) or to alert users (water processing plants) of pulses of low quality through the application of closed loop seromechanism principles.

II EXAMPLES OF AUTOMATIC WATER QUALITY DATA ACQUISITION SYSTEMS FOR POLLUTION SURVEILLANCE

Integrated multiparameter systems have been employed for continuous monitoring in pollution surveillance at many locations throughout the United States.

A Acid Mine Drainage

Multiparameter systems measuring

pH
Conductivity
Dissolved Oxygen
Oxidation-Reduction Potential
Temperature

were employed at Roaring Creek and Grassy Run streams for monitoring drainage from strip mines near Elkins, West Virginia.

B Buffalo River

1 An integrated system capable of measuring

pH
Conductivity
Dissolved Oxygen
Temperature

is planed for installation on the Buffalo River at the Ohio Avenue Bridge, Buffalo, New York

2 Buffalo Stream Improvement Corporation

Five companies had formulated this corporation for the primary purpose of employing Lake Erie waters for process applications. These industries are all located along the Buffalo River.

C Calumet Area Post Action Surveillance Program, FWPCA

Integrated systems have been installed by this project for the measurement of

pH
Conductivity
Dissolved Oxygen
Temperature

1 Indiana Harbor

2 Calumet River

D Detroit River

An automatic water quality monitoring system has been installed near Grosse Ile on the western shore of the Detroit River for measuring

Conductivity
Dissolved Oxygen
Temperature

E John H. Kerr Dam and Reservoir

An integrated two-parameter system has been installed at this dam on the Roanoke River for the measurement of Dissolved Oxygen and Temperature. This system measures the discharge of the dam through the application of remote sensors which are mounted at the end of a dock. The system provides both analog and digital output.

F Potomac River

The Potomac River Pollution Surveillance System is comprised of four water quality instrumentation systems integrated into a network having both 8-channel paper tape and typewritten output centrally located at the FWPCA headquarters.

1 Stations

a Arlington Bridge

Single-level sampling is employed for the following measurements:

pH
Conductivity
Dissolved Chlorides
Dissolved Oxygen
Temperature
Solar Radiation

b Wilson Memorial Bridge

Single-level sampling is employed for the measurement of:

Dissolved Chlorides
Dissolved Oxygen
Temperature

c Fort Washington and Indian Head

Two-level sampling is employed at these two stations for the measurement of

Dissolved Chlorides
Dissolved Oxygen
Temperature

Those stations which employ two-level sampling include independent parametric systems and independent intake systems for each level. The first sample is drawn approximately 10 feet from the surface, and the second level is taken approximately 30 feet below the surface.

Slide 21

2 Telemetry

The telemetry system employs Schedule 1 transmission lines and carries 62.5 milliamperes of current which is interrupted on a frequency basis in proportion to the amplitude of each parametric measurement. An interruption of 15 pulses per second represents full-scale deflection, and an interruption of 5 pulses per second represents an output of zero for any given measurement. The transmission line service is provided through the efforts of AT&T and the Chesapeake and Potomac Telephone System.

3 Central Station

The central station for the Potomac River Pollution Surveillance System is comprised of several functions:

Digital clock
Programmer
Receiver
Frequency-to-millivolt converter
Analog-to-digital converter
Output mechanism including 8-channel
  paper tape and IBM Selectric
  Typewriter

The operation of the system is based upon hard-wire programming, but its flexibility permits change in output format and sequence of station address.

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This outline was prepared by A.F. Mentink, Chief, Instrumentation Development, Analytical Quality Control Laboratory, Division of Research, WQO

Descriptors: Data collection, Monitoring, networks, Stations, Telemetry, Water Analysis

EXTRACTION p-VALUES OF PESTICIDES

I INTRODUCTION

A The extraction p-value introduced by Beroza and Bowman in 1965 is based on the partition of a compound between two immiscible phases.

B The principle of partition is well known and the p-value determination is a good practical application for the identification of pesticides.

C The p-value is defined as the fraction of a solute partitioning into an upper phase of an equal volume two-phase system.

D The p-values have been determined for 131 pesticides in six binary solvent systems.

II GENERAL PROCEDURE

A A 5-ml aliquot of one of the upper phase solvents containing a given pesticide is analyzed quantitatively usually by E.C.G.C. To a second aliquot (5 ml) in a 10-ml graduated centrifuge tube, an equal volume of a lower phase solvent is added and the tube shaken for one minute. The upper phase is analyzed, quantitatively. The ratio of the second analysis to the first is the p-value. (The amount of pesticide in the upper layer divided by the total amount of pesticide determined in the first analysis.)

B Six solvent systems have been used and the p-values given. The solvent systems have been chosen to give the widest range of p-values.

C These systems are commonly used in pesticide analysis and often the solvents used for extraction do not have to be altered for p-value determination.

III NOTES ON THE PROCEDURE

The solvent systems used by Bowman and Beroza were selected as the best all around systems by a process of elimination from a larger original number of systems. The systems finally decided on are listed here:

Hexane/Acetonitrile
Hexane/90% Dimethylsulfoxide
Isooctane/85% Dimethylformamide
Isooctane/Dimethylformamide
Heptane/90% Ethanol
Isooctane/80% Acetone

IV ADVANTAGES

A The p-value determinations are practically independent of concentration and the presence of extract interferences.

B The sensitivity is in the nanogram range.

C The method adds another confirmation of identity by measuring another physical property.

D It is simple and quick giving results in a matter of minutes.

E The procedure can be used for mixtures of insecticides.

F No special equipment is required.

G Pesticides having the same retention time on a G.C. column can be quickly distinguished using p-values and in many cases valuable time saved by eliminating the need for G.C. column changes.

H Other solvent systems may be substituted for the six originally used.

I If an unknown compound is being analyzed, it is not necessary to know the exact amount, only the relative amounts.

V DISADVANTAGES

Some compounds are reactive in a specific solvent system or react when injected into the G. C. unit and these p-values are not reliable.

VI APPLICATIONS

The following table shows a practical application on an actual sample of fish blood:

p-VALUES

Sample	Hexane / 80% Acetone	Isooctane / 80% Acetone	Hexane / Acetonitrile
Blood Extract	0.95	0.85	0.26
Aldrin	0.96	0.98	0.73
Parathion	0.75	0.76	0.044
Malathion	0.52	0.46	0.042

Aldrin, parathion, and malathion all had identical retention times on the G. C. column used. The gas chromatogram of the sample showed a peak at the same retention time, but with p-values was shown to be none of the three insecticides.

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This outline was prepared by H.W. Boyle, Physical Science Technician, Waste Identification and Analysis Activities, Advance Waste Treatment Research Laboratory, 4676 Columbia Parkway
Cincinnati, OH 45226

Descriptors: Chemical Analysis, Pesticide Residues, Pesticides, Separation Techniques, Solivent Extractions, Water Analysis

INSTRUMENTAL ANALYSIS OF PHENOLS AND MERCAPTANS BY ELECTRON CAPTURE GAS CHROMATOGRAPHY

I INTRODUCTION

A Foreword

To aid in monitoring waterway, sensitive, rapid and more specific methods are needed for measuring traces of mercaptans and phenols that adversely affect the palatability of water. In addition, certain types of phenols, e.g., dinitrophenol, pentachlorophenol, have certain tolerance limits suggested because of their toxicity. Because of the multitude and complexity of our manufacturing processes, one can assume that the measurement for specific compounds will be more difficult to conduct in order to establish responsibility for violations of water quality standards and to secure abatement of pollution.

This procedure is developed for the qualitative and quantitative weak acid analyses of grab samples at sensitivities 500 to 1000 times greater than present methods. In addition, this method eliminates the tedious and time-consuming cleanup procedures that are characterized by inherent losses and decomposition.

B The entire procedure for the analysis involves collection, extraction, derivative preparation and gas chromatographic analyses.

C Equipment

1 All glassware are cleaned in a detergent water solution, rinsed well with distilled water, and then heated at 1000°F in a mechanical convection oven for thirty minutes. After cooling, all orifices of the clean glassware are wrapped and sealed with fresh aluminum foil.

2 Extractions may be carried out with a liter separatory funnel or a semi-automatic extractor using 15% methylene chloride in hexane. (Certified D-37 Methylene chloride may be obtained from Fisher Scientific Company. Purified hexane, distilled in glass, is purchased from Burdick and Jackson Laboratories, Muskegon, Michigan.)

3 Solvents may be removed in a rotating vacuum evaporator or in an all-glass distillation system attached to the building vacuum system or to a mechanical vacuum pump. The latter system consists of a round bottom flask fitted to a simple Claisen distillation head with a thermometer well, water condenser, adaptor, distillate receivers of the "udder" type, and a dry ice trap. The system is then attached to a building vacuum line or a mechanical pump.

4 Derivatives are prepared in an all-glass equipment, consisting of a water condenser fitted with 19/38 joints and capped with a tin foil dust trap, and a round bottom flask of 100 to 250 ml capacity.

5 A Perkin-Elmer Gas Chromatograph, Model 811, with tritium foil Electron Capture Detector of parallel plate design is used. An 8 foot by 0.25 inch aluminum tubing containing a 50-50% mixture of 5% F.S. 1265 and 3% D.C. 200 supported on Chromosorb P of 60-80 mesh size is employed at 200°C. Nitrogen flow is 40 ml per minute. The sensitivity is set at an attentuation of x-50 and x-100, while the power supply is operated on DC 10 volts.

The gas chromatographic column, through previous conditioning with injections of aldrin, endrin, etc., and through several months use for pesticide analyses, yields excellent separations and is recommended for the analyses.

II PROCEDURE

A Collection

Collect the water sample in a liter sample jar. Cap the completely filled jar with a screw cap fitted with a Teflon liner. Preserve, prior to extraction, the samples in a refrigerator.

B Extraction

To a liter separatory funnel, add 65 ml of 15% dichloromethane in hexane. Of the 1 liter of water sample, take and introduce a 700 ml portion into the separatory funnel. Add three drops of concentrated hydrochloric acid so that the water sample is slightly acid. Tilt the separatory funnel back and forth for several minutes to extract the organic materials. Set the funnel with its contents on a ring stand in a well-ventilated hood. After the lower layer is drawn off, add an additional 65 ml of the 15% dichloromethane-hexane mixture. Repeat the extraction process until 200 ml of extract are collected. Wash once with 15 ml of organic-free, distilled water. Collect the lower dichloromethane-hexane layer in a 250 ml round bottom distillation flask after passage through a short-stemmed funnel fitted with fluted filter paper that is filled with several grams of anhydrous sodium sulfate. Remove the solvent with aid of a rotating vacuum flask in which the contents of the flask are heated on a water bath at 50°C to 65°C. Collect the condensate in a dry ice trap which is attached to a mechanical vacuum pump or a building vacuum line. For the more volatile mercaptans, add 2 ml of 0.86 N alcoholic KOH prior to the solvent removal step. (NOTE: An all-glass distillation equipment may be used in place of the rotating vacuum evaporator.)

C Conversion to a Derivative

Add to the dried residue 8 ml of 0.8N alcoholic KOH, 20 ml of distilled absolute ethanol, and 10 ml of acetone. Stir the mixture with the aid of a magnetic stirring bar placed in the flask mounted above the stirring motor. After 5 minutes, shut the stirrer off and add the equivalent of 20 mg of the pentafluorobenzyl bromide reagent contained in 2 ml of alcohol. (Prepared in the hood by diluting 1.00 gram of reagent to 100 ml.) Stir the mixture for five minutes. Place the flask and contents on a water bath. To the flask, attach upright a water condenser fitted with a 24/40 joint. Cover the top of the condenser with aluminum foil fitted tightly over the top of the condenser.

Permit the reaction mixture to reflux for two hours. Cool. Rinse the insides of the condenser with 10 ml of 15% dichloromethane-hexane mixture. Remove, and fit the flask containing the reaction mixture to a rotating evaporator or to an all glass vacuum distillation system in which the distillate may be collected and condensed in a dry ice condenser.

Extract the dried residue with 90 ml of 15% dichloromethane in hexane and 20 ml of water in the distillation flask. Rotate thoroughly the flask and contents to extract the material at the sides of the flask. Transfer the extract layer and the water layer to a separatory funnel. Shake gently for five minutes. Permit the two phases to separate. Filter the lower extract through anhydrous sodium sulfate (3 grams) contained in a fluted filter paper held in a short-stem glass funnel. Determine the pH of the aqueous wash (top layer) which should be alkaline. Collect the filtrate in a 250 ml round bottom flask. Wash the filter paper and Na_2SO_4 with 10 ml of 15% CH_2Cl_2 in hexane. Remove the solvents in the manner described previously. Weigh the dried residue and then dilute to 100 ml with 3% acetone in hexane. Dilute 1 ml of the initial dilution to 100 ml with 0.3% acetone in hexane. From this solution, inject several microliters of solution into the gas chromatograph.

III ANALYSES - ELECTRON CAPTURE GAS CHROMATOGRAPHY

A Identification

For the identifications of phenols, as ethers, make a 2 µl, 4 µl, and 6 µl injections at proper intervals. Repeat this procedure two more times. Determine the injection volume - response curve. Dilute a measured 80 mg of known pentafluorobenzyl phenyl ether to an equivalent volume of 100 ml with 3% acetone in hexane. Dilute 1 ml of the initial solution to 100 ml with 0.5% acetone in hexane. From this second dilution, inject a 4 µl volume or an appropriate volume into the electron capture gas chromatograph. Repeat the injection two times and calculate the average retention time. Divide the average of the retention times of each peak from the unknown by the average of the retention time of pentafluorobenzyl phenyl ether. Compare the relative retention time of each unknown ether with those listed on Table I. Check the absolute retention time of the unknown with that of the prepared known derivative for comparison. The two retention times should check.

TABLE I

PROPERTIES OF ETHERS AND THIOETHERS

Pentafluorobenzyl Aryl (or Alkylthio) Ether	M.P.°C	R.I. 24° n_D	Yield %	Retention Time (Min.)	Relative Retention (To Phenolic Ether)
(1) t-Butylthio		1.4744	99	1.61	0.67
(2) n-Butylthio		1.4676	98	1.85	0.77
(3) Phenyl	75- 76		96	2.40	1.00
(4) 2-Tolyl	78- 79		88	2.72	1.13
(5) 4-Tolyl	74- 75		85	2.91	1.21
(6) 2, 4 Xylyl	50- 52		94	3.27	1.36
(7) 2-Chlorophenyl	91- 93		99	3.74	1.56
(8) 4-Chlorophenyl	66- 67		94	4.11	1.72
(9) 2, 4-Dichlorophenyl	73- 74		86	5.91	2.46
(10) n-Decylthio		1.4662	86	7.36	3.06
(11) n-Undecylthio		1.4665	100	9.68	4.03
(12) Naphthyl-2	132-133		84	10.46	4.35
(13) n-Dodecylthio		1.4668	96	12.91	5.37
(14) 2-Nitro, 4-Chlorophenyl	99- 99.5		92	16.26	6.76

B Quantitative Analyses

Make the calculations of the amount of unknown present in each identified peak by comparing the area under the known peak and the amount injected for the known peak versus the area under the identified peak and the amount injected for the unknown.

$$\frac{A}{W} = \frac{Ax}{Wx}$$

(Where A is area of curve obtained by injecting the known derivative, W is the weight injected of the known derivative (nanograms), and Ax is the area of the curve obtained by injecting the derivative of unknown origin. Wx is the weight of the derivative of unknown origin.) Calculate the weight of the identified derivative by solving for Wx:

$$Wx = \frac{W \cdot Ax}{A}$$

Then multiply Wx (nanograms) by the dilution factor, e.g. 10,000. Calculate the amount of the phenol by using the fraction formed by the molecular weight of phenolic compound divided by the molecular weight of the derivative (ether of the phenolic compound).

Thus for paracresol, use the following calculations:

$$\frac{Wx\ (\text{nanograms}) \times 10^4 \times 108.14}{0.7 \times 288.23} =$$

weight of the paracresol in the original grab sample per liter.

C Mercaptans

Determine the mercaptans as pentafluorobenzyl alkyl (aryl) thioethers in a similar manner with use of the sulphur cell microcoulometric titration gas chromatography.

D Interference

None known at present time.

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This outline was prepared by F.K. Kawahara, Organic Chemist, Analytical Quality Control Laboratory, 1014 Broadway, Cincinnati, OH

Descriptors: Chemical Wastes, Gas Chromatography, Instrumentation, Phenols, Water Analysis

*Mention of products by manufacturers is for identification only and does not imply endorsement by the Federal Water Pollution Control Administration of the U.S. Department of the Interior.

SPECIAL INSTRUMENTAL METHODS - MASS SPECTROMETRY AND NMR

I INTRODUCTION

Mass spectrometry and nuclear magnetic resonance spectrometry (NMR) are now widely used in organic analytical chemistry, but have only begun to find application to the analysis of chemical pollutants in the aquatic environment. Now that the cost, and in some cases the complexity of these instruments have decreased, they will undoubtedly become quite important in water pollution analysis during the next few years. This is especially true of mass spectrometry, and the majority of this lecture time will be devoted to examples of applications of this instrumental method to water pollution problems encountered at the Southeast Water Laboratory. Examples of NMR applications will also be given, as well as a brief discussion of the cost and manpower requirements, sample requirements, operating principles, and informational output of both methods. Inorganic mass spectrometry, a very useful method for elemental analysis of water samples, will not be discussed since the operating techniques and sample requirements are quite different from those for organic mass spectrometry.

II MASS SPECTROMETRY

A Economic factors

1 Mass spectrometers range in price from \$20, 000 to \$200, 000 or more. Ordinary general purpose instrumentation begins at between \$30, 000 and \$35, 000.

2 Mass spectrometers are complicated instruments and generally are "down" from 10% to 50% of the time, depending upon the make and complexity of the spectrometer. Maintenance requires money for parts and service.

3 Time is an important economic factor. To operate, maintain, and interpret spectra from a mass spectrometer of medium complexity with a reasonable work load, at least one trained person is required full time.

B Sample requirements

1 Solid samples can be handled by direct probe accessories. From 10^{-12} to 10^{-7} grams of sample are required, depending upon sample volatility and instrument sensitivity. Much information can be obtained from a sample of low purity, say 90%, and some useful information can be obtained from simple mixtures. Viscous liquids are also generally handled in the direct probe.

2 The liquids sample inlet on a mass spectrometer is generally used for volatile liquids. Sample size is between 0. 1 and 1 mg, and, here again, much information can be obtained from impure samples or in certain cases, samples in solution.

3 Gaseous samples are handled in special gas sampling flasks and inlets.

4 Gas chromatographic inlet system

The interfacing of a gas chromatograph to a mass spectrometer provides a very useful sample introduction mode. Using this technique, any compound that will chromatograph is eluted from the GC column, passes through a special sample enricher, and goes into the mass spectrometer proper. The enricher, known as a separator, removes the GC carrier gas preferentially, thus increasing the concentration of the eluted sample compound while lowering the pressure to a level suitable for the mass spectrometer. The application of this GC introduction mode to water pollution analysis is obvious. An organic extract of a water sample is chromatographed under previously established conditions, and a rapid scan (1 to 3 seconds) is made of each eluting component of the extract to produce the corresponding mass spectrum. Sample size required here is usually on the order of 0. 1 μg of each component, although useful spectra can sometimes be obtained with nanogram quantities.

C Operating principles

Regardless of which sample introduction mode is used, the sample must be in the gaseous state for the process of ionization. The sample molecules are bombarded by a beam of electrons of variable energy in the ion source (Figure 1) to produce positive

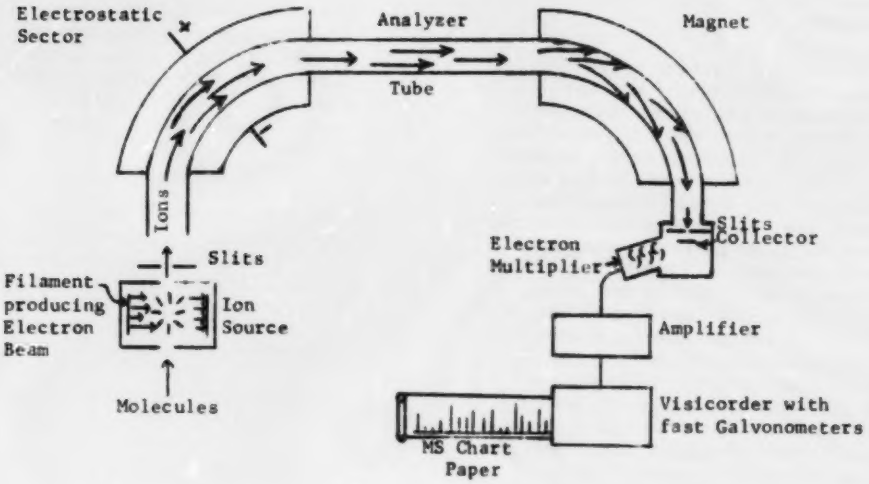
Electrostatic Sector
Analyzer
Tube
Magnet
Ions
Slits
Filament producing Electron Beam
Ion Source
Molecules
Electron Multiplier
Slits
Collector
Amplifier
Visicorder with fast Galvonometers
MS Chart Paper

Figure 1. Block diagram of a double-focusing mass spectrometer.

ions. These ions are swept through a series of slits and down the analyzer tube by a strong electrostatic field. The source and tube must be maintained at a low pressure ($<10^{-5}$ torr) to minimize collisions of the ions with each other or with air molecules. The ions pass through a magnetic field where they are deflected to different degrees based on their mass to charge ratios. By sweeping the magnetic field strength, ions of successively increasing mass are sequentially brought into focus at the collector. In a double-focusing mass spectrometer, resolution is improved by an electrostatic sector which renders the ion beam monoenergetic by velocity focusing before it arrives at the magnetic focusing sector. The electron multiplier amplifies the signal received at the collector and the resulting signal is further amplified to drive a set of galvonometers which make a trace on a photographic chart paper.

D Information output

1 All fragment ions of a molecule give rise to peaks of certain intensities with positions on the abscissa of the spectrum corresponding to ion masses (m/e values). This information constitutes the mass spectrum, and comparison with the spectra of known compounds give definitive compound identification. Figure 2 is a plot of a typical mass spectrum, that of bis-(2-ethylhexyl) phthalate, compared with that of a compound extracted from the Tennessee River. They are practically identical.

2 The molecular weight of a compound can often be obtained from its mass spectrum. The fragment ion of greatest mass in most cases corresponds to the molecular weight of the compound (Figure 2, $M^{+}=390$), having been created by loss of only one electron during electron bombardment.

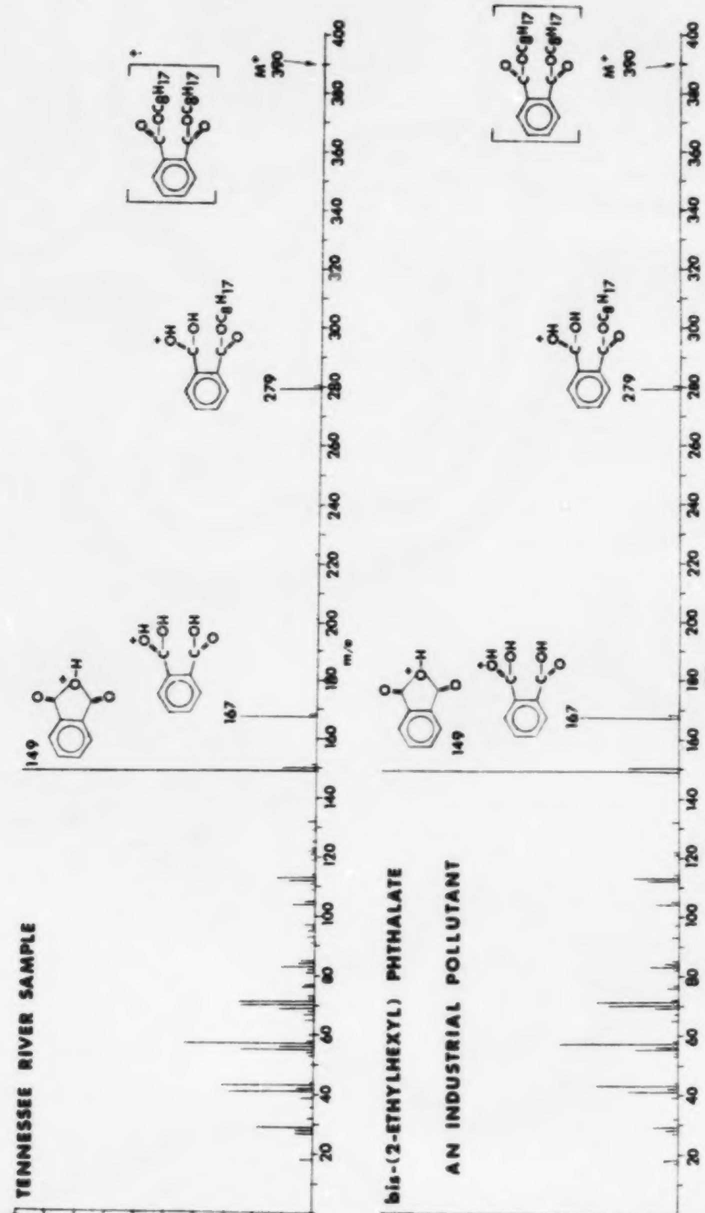
TENNESSEE RIVER SAMPLE
bis-(2-ETHYLHEXYL) PHTHALATE
AN INDUSTRIAL POLLUTANT
Relative Intensity
m/e
149
167
279
M+ 390
OC8H17

Figure 2

3 The masses of important fragment ions indicate the empirical formulae of the ions and hence give clues as to structures of parts of the molecule (Figure 2).

4 Isotope peaks are helpful in determining if a molecule contains chlorine, bromine, sulfur, mercury, and other atoms which have more than one highly abundant naturally occuring isotope.

5 High resolution mass spectrometry is capable of providing almost the exact mass (to four decimal places) of the parent ion and of the important fragment ions from the molecule. This degree of accuracy of mass determination eliminates all but one or two possible empirical formulas for these ions, thus providing very important clues to the molecular structure.

III EXAMPLES OF APPLICATIONS OF MASS SPECTROMETRY TO WATER POLLUTION PROBLEMS

(Corresponding spectra will be projected and discussed.)

A The identification of S, S, S-tributyl phosphorotrithioate in Charleston Harbor, S. C., depended to a large extent on molecule weight determination by mass spectrometry.

B The identification of phenyl mercuric chloride, believed to be the cause of a fish kill in Boone Lake, Tennessee, involved the observation of mercury and chlorine isotope peaks in the mass spectrum.

C Diazinon was separated from a Tombigbee River water extract by thin layer chromatography, removed from the adsorbent, and analyzed by mass spectrometry.

D The analysis of petroleum rocket fuel residues extracted from water illustrate the applicability of mass spectrometry to oil pollution analysis.

E Bis-2-(ethylhexyl) phthalate was identified as a pollutant in the Tennessee River with the aid of mass spectrometry (Figure 2).

F An actinomycetes metabolite, responsible for earthy taste and odor problems in an Ohio municipal water supply, was identified as 2-methylisoborneol. The mass spectral fractionation pattern of the unknown compound was important in the elucidation of its structure.

G Nonylphenol has been extracted from a treated woolen mill effluent, collected upon elution from a gas chromatographic column, and identified by mass spectrometry using the direct probe accessory.

H Several terpenes and terpene derivatives in the treated effluent of a kraft paper mill have been identified by combination gas chromatography-mass spectrometry (GC-MS). The complexity of the gas chromatogram illustrates both the great problems encountered in waste characterization and the beauty of the GC-MS technique; whereby, many of the individual components can be identified.

I Three resin acids and several fatty acids have been identified in the effluent and in the foam downstream from a paper mill. The methyl esters of the acids were prepared by reaction with diazomethane, and the volatile esters were analyzed by the combined GC-MS technique.

IV NUCLEAR MAGNETIC RESONANCE SPECTROMETRY (NMR)

A Economic factors

1 NMR instrumentation ranges in price from about $20,000 for a "desk-top" low resolution (60 MHz) spectrometer to $85,000 for a 100 MHz instrument with common accessories.

2 NMR equipment is not as mechanically complex as mass spectrometry instrumentation; down time is generally only 10% to 20% and a full time operator is not necessary for a reasonable work load. However, an experienced operator is necessary for optimum output.

B Sample requirements

1 Conventional NMR samples must be in the liquid phase, either neat or in solution. Solvents must be deuterated or aprotic (e.g., CCl_4, CS_2) since any protons present absorb energy to give spectral interferences. Milligram quantities of sample (in solution) are required except that with particularly sensitive instrumentation 0.1 mg can sometimes give good spectra, and with

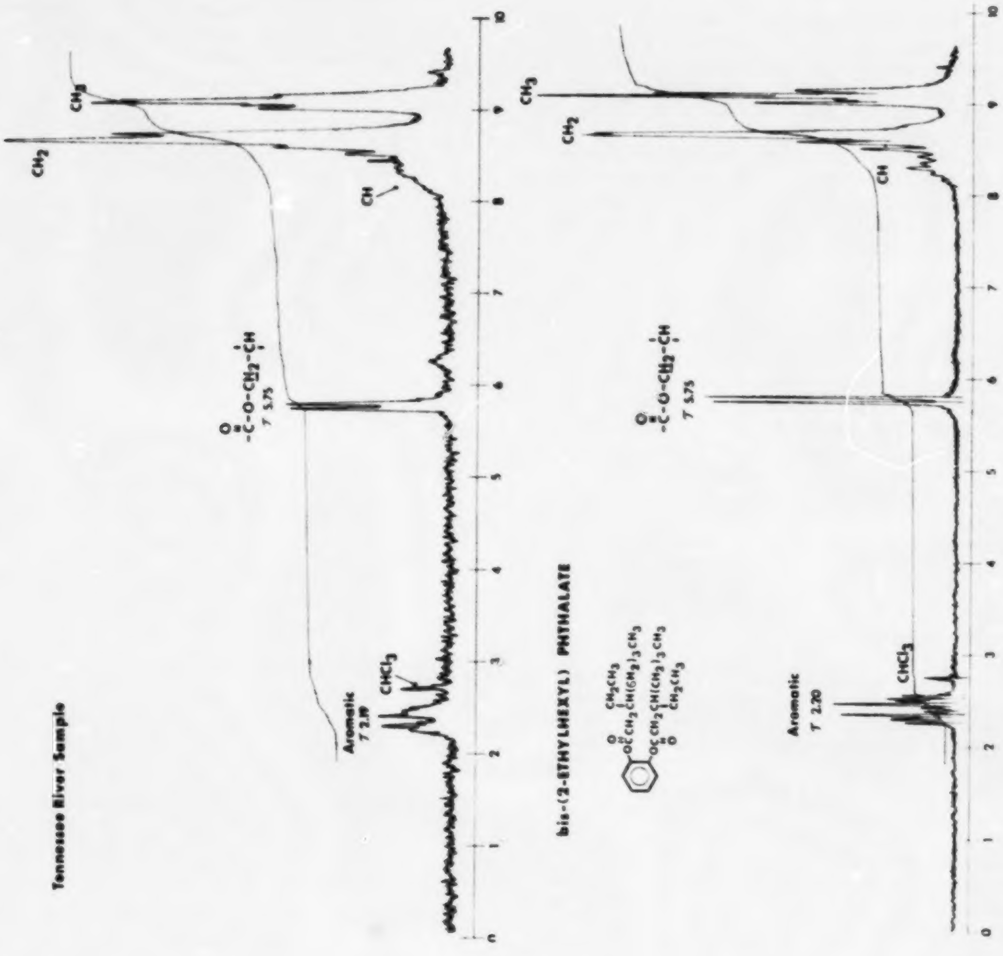
Tennessee River Sample
Aromatic
τ 2.19
CHCl$_3$
-C(=O)-O-CH$_2$-CH
τ 5.75
CH
CH$_2$
CH$_3$
bis-(2-ETHYLHEXYL) PHTHALATE
Aromatic
τ 2.20
CHCl$_3$
-C(=O)-O-CH$_2$-CH
τ 5.75
CH
CH$_2$
CH$_3$
0
1
2
3
4
5
6
7
8
9
10

Figure 3

time-averaging computerization to enhance the signal to noise ratio, less than 10 micrograms is sometimes sufficient. A purity of 90% is usually adequate to give useable spectra, and simple mixtures can often yield useful information.

2 A lack of sensitivity (a factor of 10^3 to 10^6 times as much sample is required as for mass spectrometry) and the longer time required for analysis are the principal disadvantages of NMR. The method is extremely valuable; however, in detailed structural elucidation, providing information as to the types and environments of hydrogen atoms which cannot be obtained by any other instrumentation.

C Information output

Hydrogen nuclei absorb energy in the radiofrequency range at certain frequencies depending on their environment. Thus, signals at certain frequency areas of the spectrum are characteristic of methyl, methylene, aldehydic, aromatic, etc., protons. (Figure 3). The NMR is capable of integrating these signal areas relative to each other; therefore, allowing calculation of the number of each type of proton in the molecule. In the case of simple mixtures, this feature often allows the calculation of percentage composition of the components.

In addition to absorbtion of radiofrequency energy, nuclei with magnetic moments, such as hydrogen, phosphorus, and carbon-13, interact with each other when in the same proximity to cause "splitting" of the proton signal peaks into a larger number of smaller peaks (Figure 3). This is known as "spin-spin coupling" and is a very informative element of the NMR method. Splitting rules allow the calculation of the number of peaks resulting from the coupling of one proton with other adjacent protons. Thus, the degree of splitting of a particular hydrogen signal is indicative of the protonic environment of the hydrogen atoms, and this allows important deductions to be made regarding the structure of the molecule.

D Examples of applications of NMR to water pollution problems

1 The NMR spectrum of a phthalate ester isolated from the Tennessee River provided the prime evidence for the detailed structure of the molecule, especially of the 2-ethylhexyl moities. It was later shown that this spectrum matched that of bis-(2-ethylhexyl) phthalate (Figure 3).

2 The NMR spectrum of the actinomycetes metabolite extracted from an Ohio municipal water supply showed the compound to have four tertiary methyl groups.

3 The frequency of the methylene absorption signals of the NMR spectrum of a toxic compound isolated from Charleston Harbor, S. C., indicated the compound to be the phosphate derivative of a phosphite ester present in an industrial effluent discharging into the harbor.

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This outline was prepared by Arthur W. Garrison, Research Chemist, Southeast Water Laboratory, WQO.

Descriptors: Chemical Analysis, Instrumentation, Mass Spectrometry, Nuclear Magnetic Resonance, Spectrophotometry, Water Analysis

DOSAGE AND PROCESS CONTROL

The quality of treated water and the chemicals costs for its production are dependent on proper control of the coagulation-flocculation processes. Laboratory and in-plant control are essential to proper coagulation treatment for two basic reasons: First, plants which employ coagulation usually treat surface waters which may be subject to wide variations in quality and are much more likely to be contaminated than well supplies. Second, the best and most economical treatment of such waters cannot be predicted from known relationships but must be checked by one or more testing or monitoring techniques. The coagulation and flocculation of water is influenced by a number of interrelated factors such as pH, color, turbidity, mineral content and composition, temperature, the duration and degree of agitation during flocculation, and finally, the characteristics of the coagulant or flocculation aid in use. Unfortunately, the optimum conditions for coagulation cannot be determined from the results of a chemical and physical analysis of the water, but must be determined empirically. The type and frequency of tests depend largely on the character of the raw water and on the plant design. Where possible, laboratory investigations of treatment should begin before the plant is designed and constructed, since operation and control can be simplified if the plant is designed specifically to handle the particular raw water, especially if that water is subject to wide variations in quality. Because each water plant may require its own specialized combination of tests and methods for performing these tests, the subject of dosage and process control will be treated here only in a general way.

I THE JAR TEST

The single most widely used test to determine dosage and other parameters is the "jar test." The equipment for this test and the directions for the proper performance have been published. The jar test attempts to simulate the full-scale coagulation-flocculation process and has remained the most common control test in the laboratory ever since its introduction in 1918. Since the intent is to simulate an individual plant's conditions it is not surprising that there has been no standardization of the test. The jar test as variously performed does, however, have some elements of conformity. In its essentials, the jar test simply consists of a series of sample containers, the contents of which can be stirred by individual mechanically operated stirrers. Water to be treated is placed in the containers, and the treatment chemicals are added while the contents are being stirred. The range of conditions, for example pH and coagulant dosages are selected to bracket the anticipated optima. After a short, 1 - 5 minute period of rapid stirring to ensure complete dispersion, the stirring rate is decreased and flocculation is allowed to continue for a variable period, 10 - 30 minutes or more, depending on the simulation. The stirring is then stopped, and the floc are allowed to settle for a selected time. The supernatant is then analyzed for a variety of parameters.

The interpretation of the results of this test requires some determinative measure of the flocculation. A number of approaches have been used: (1) visual evaluation of floc size, (2) visual or photometric measurement of supernatant clarity or color, (3) time of appearance of first floc, (4) chemical determination of residual coagulant in supernatant, Figure 1, (5) rate of settling of floc, Figure 2, (6) measurement of the electrophoretic mobility (zeta potential) of the particles, and (7) more recently, the filterability characteristics either through paper, membranes or through miniaturized sand filters, Figure 3.

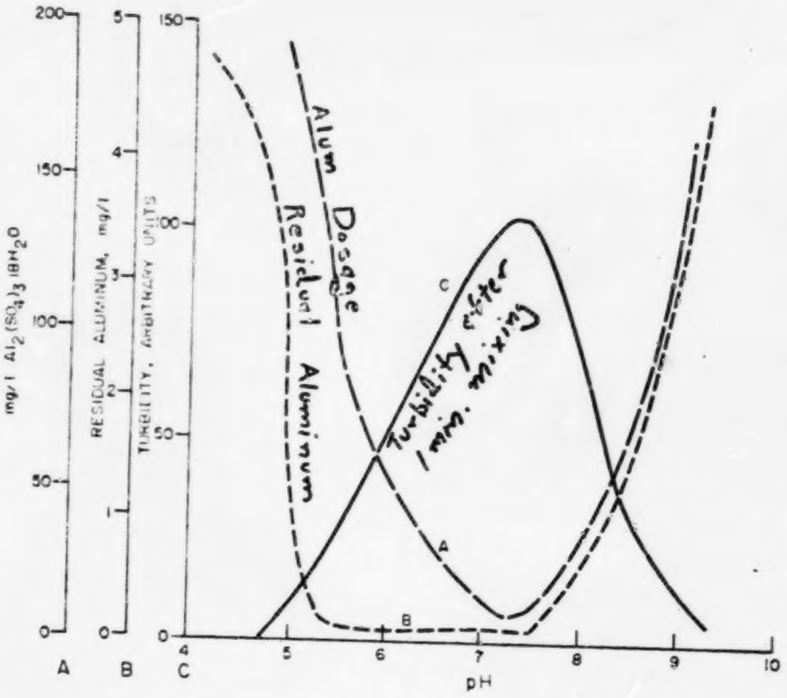
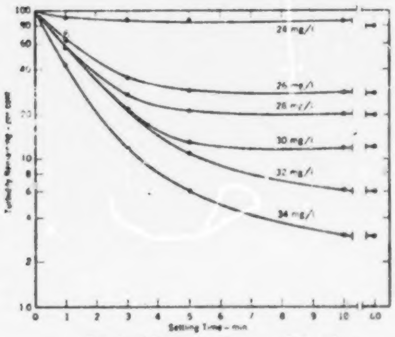


FIGURE 1
COMPARISON OF THE EFFECTS OF pH

Since the objective of coagulation-flocculation is to destabilize the particles so that removal can be obtained by sedimentation and filtration, residual turbidity measurement has been a most important parameter. Turbidity, as the word is understood in the water field, is simply an optical property of the suspended particles in the water, and it is not a direct assessment of the quantity present. Since the measured optical properties of particles vary depending on their size distribution, refractive index, concentration, angle of observation with respect to the light source and on the instrument geometry, it is not surprising that numerical values for turbidity may not be compared between plants. The problem is further complicated because of the lack of a generally accepted reference turbidity material. In the usual determination of turbidity, the light scattered by the particles in the water is compared with that scattered by a dilute clay suspension which in turn is standardized by the Jackson Candle method. The problem here is that the Jackson Candle method cannot be used for turbidities less than 25 units, and that clay suspensions are neither standard nor reproducible among laboratories. There are ongoing efforts to standardize this test particularly



Effects of Increasing Amounts of Coagulant

Ferric sulfate was added to six jars in amounts shown. Original turbidity of the water was 95 units. Dots indicate sampling points.

FIGURE 2

for the low turbidities of interest to the water plant. The direction of the effort is toward a standardized instrument geometry, and some standardized reference material.

II ZETA POTENTIAL MEASUREMENT

Particles suspended in water carry electrical charges at their surfaces. For particles in natural waters, the charge is usually negative as illustrated schematically in Figure 4. These electrical charges may arise through ionization of atoms at the particle surface, or particles may acquire the charge through adsorption of ions, particularly hydroxyl ions, from the water itself. These ions are tightly bound to the surface of the particle and attract ions of opposite charge from the mixture of positive and negative ions in the water. This layer of oppositely charged ions is also tightly held near the particle. As the force of attraction of ions decreases with increasing distance from the particle a diffuse layer is formed consisting of a varying mixture of positive and negative ions extending to such a distance from the particle that the mixture of ions is now similar to the composition present in the water. There is a plane of shear representing the portion of bound water and ions that will remain attached to the particle as it moves through the suspending liquid.

A consequence of the electrical double layer surrounding each particle in water is to create areas of electrical potential in a bulk solution that nominally has a zero potential. The electrostatic work required to transport a unit charge from this bulk solution through the phase boundaries of each layer surrounding a particle to any point measures the potential at that point. The potential increases sharply with increasing approach to the primary particle surface. The potentials of interest are illustrated in Figure 4. One is at the particle surface itself and measures the total potential of the double layer and is referred to as the Nernst potential. Another is at the boundary between the Stern diffuse layers and is called zeta potential (ζ). The zeta potential is interesting and important because unlike the Nernst potential an approximate zeta potential can be measured experimentally quite easily. It should be pointed out, however, that the zeta potential does not measure the total potential but rather a portion of it. It does not include the Stern layer.

The net effect of the existence of electrical double layers around particles is to inhibit the close approach of particles to each other and hence confers stability to the suspension. Both the thickness of the double layer and the surface charge density are sensitive to concentration and valence of the ions in solution, hence stability of the suspension can be markedly affected by adding suitable ions to the solution. The metal coagulants, alum, ferric sulfate, etc., added during treatment reduce these negative charges.

Measurement of zeta potential is another procedure useful for control of the coagulation process and for evaluating the results of the jar test. Zeta potential of particles is never determined directly, but is calculated from a measurement of particle electrophoretic mobility. In the usual procedure, a special apparatus is used with which the behavior of particles can be observed microscopically. Two such devices are shown in Figure 5. Under the influence of an applied voltage;

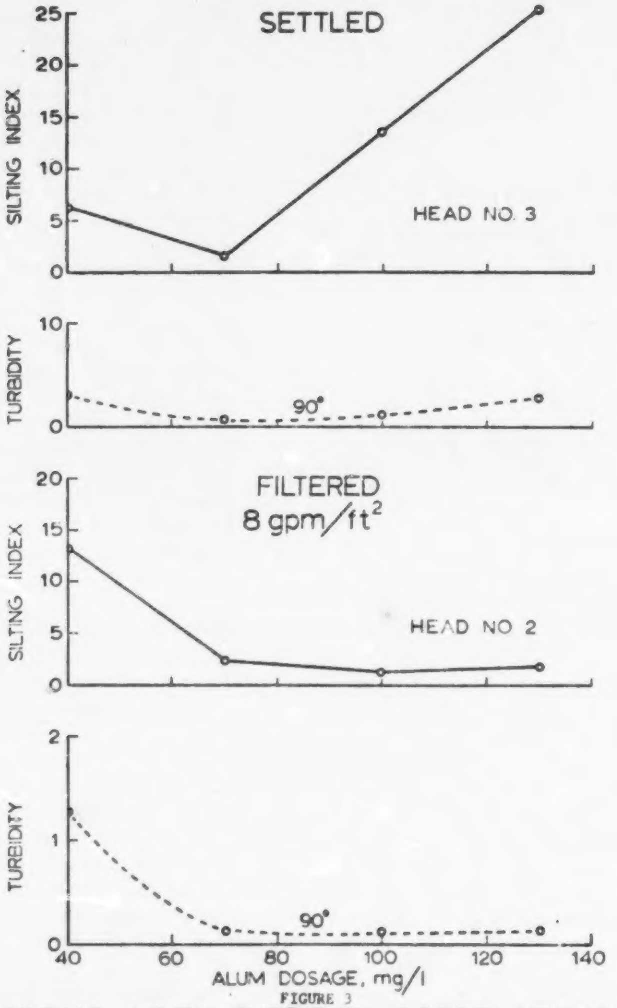


FIGURE 3

SILTING INDEX-TURBIDITY CORRELATIONS

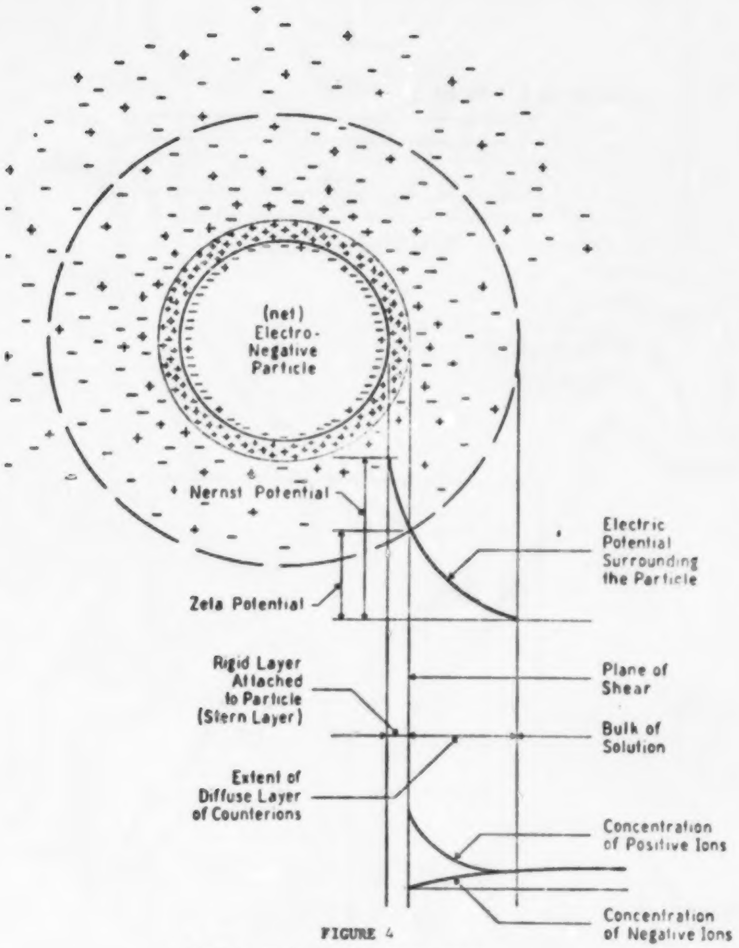

(net) Electro-Negative Particle
Nernst Potential
Electric Potential Surrounding the Particle
Zeta Potential
Rigid Layer Attached to Particle (Stern Layer)
Plane of Shear
Bulk of Solution
Extent of Diffuse Layer of Counterions
Concentration of Positive Ions
Concentration of Negative Ions

FIGURE 4

CONCEPT OF THE ZETA POTENTIAL

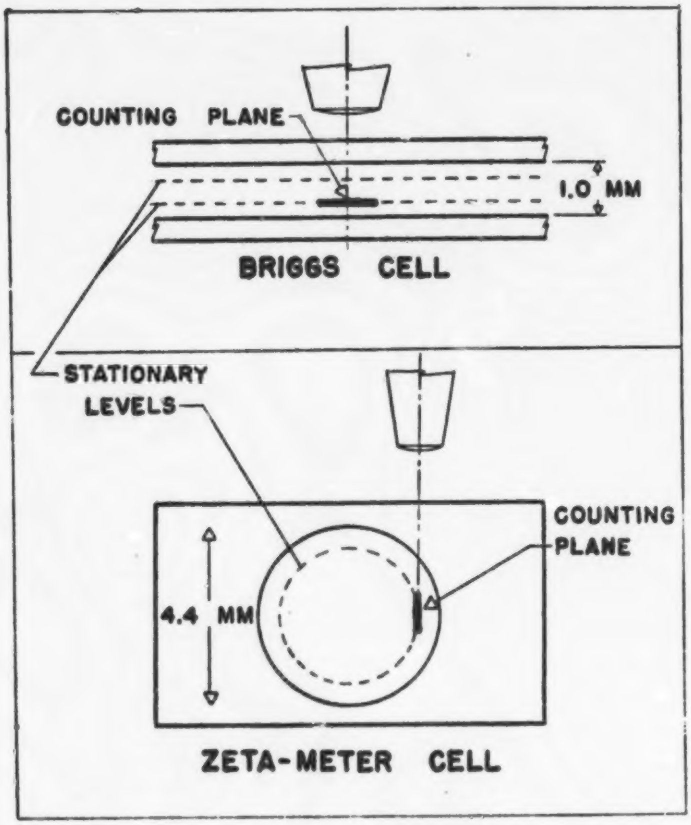

COUNTING PLANE
1.0 MM
BRIGGS CELL
STATIONARY
LEVELS
COUNTING
PLANE
4.4 MM
ZETA-METER CELL

FIGURE 5

SCHEMATIC OF CELL INTERIORS

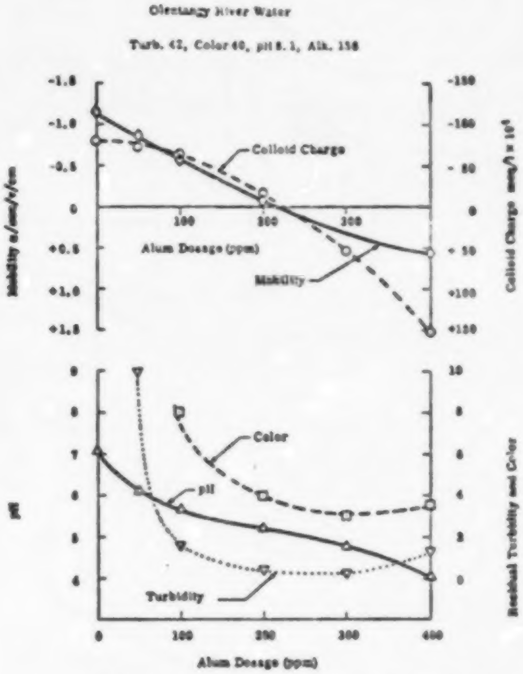
the charged particles will migrate to the poles of opposite polarity; the particle velocity can be measured by noting the time that it takes particles to traverse a known distance. From these and other data it is possible to calculate both electrophoretic mobility and zeta potential. The numerical value of the zeta potential that indicates optimum coagulation varies with the type of colloid to be removed from the water. Removal of organic colloids, such as color, is generally obtained at zeta potentials near zero, whereas optimum clay turbidity removals are obtained when zeta potentials are slightly negative ranging from -10 millivolts to perhaps -5 millivolts. But since a water may very likely contain a heterogeneous mixture of colloid types, the precise zeta potential which signals a good coagulation must be established for each water by other methods such as the jar test or by correlation to actual plant performance.

III COLLOID TITRATION

Electrophoretic mobility measurements determine the magnitude of repulsive forces of particles in water. They do not directly indicate the coagulant requirements unless a series of measurements are performed on a number of samples dosed with different quantities of coagulant. In colloid titration techniques, a single sample is dosed with coagulant until particle charges are neutralized, and an indicator which is sensitive to colloid charge changes color, Figure 6. The procedure is rapid but is subject to certain interferences. A streaming current detector, Figure 7, has been successfully used as an endpoint indicator with cationic polymers.

IV FILTERABILITY TECHNIQUES

Shull has determined a filterability number which is defined as the time required to filter a sample of supernatant from a specified jar test through a membrane filter divided by the time required to filter the same volume of distilled water under the same conditions. The filterability number is related to the alum dosage required by the plant, Figure 8.



- Relationships between particle mobility, colloid charge, turbidity, color, and pH for Olentangy river water.

FIGURE 6

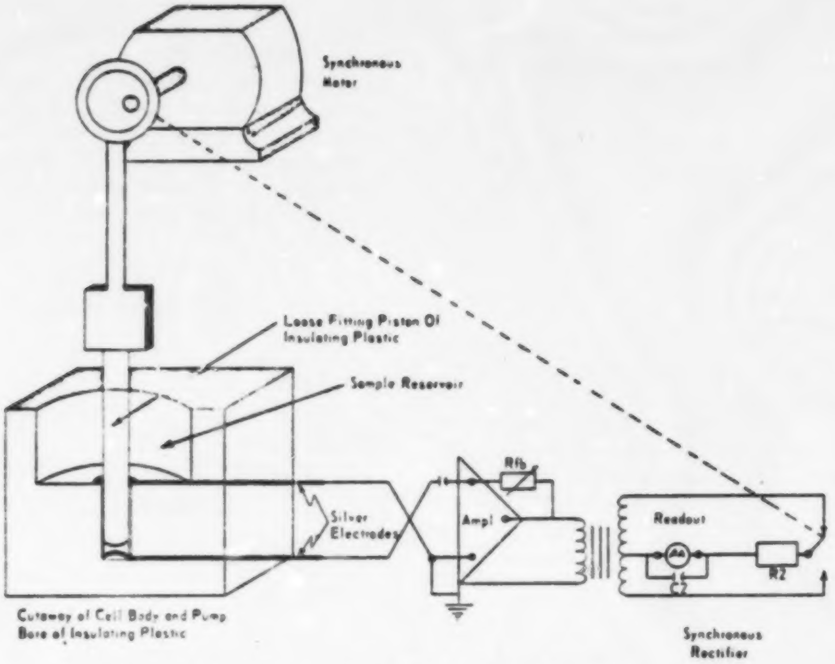

Synchronous Motor
Loose Fitting Piston Of Insulating Plastic
Sample Reservoir
Silver Electrodes
Rfb
Ampl
Readout
AA
R2
C2
Cutaway of Cell Body and Pump Bore of Insulating Plastic
Synchronous Rectifier

FIGURE 7. SIMPLIFIED DIAGRAM OF SCD INSTRUMENT

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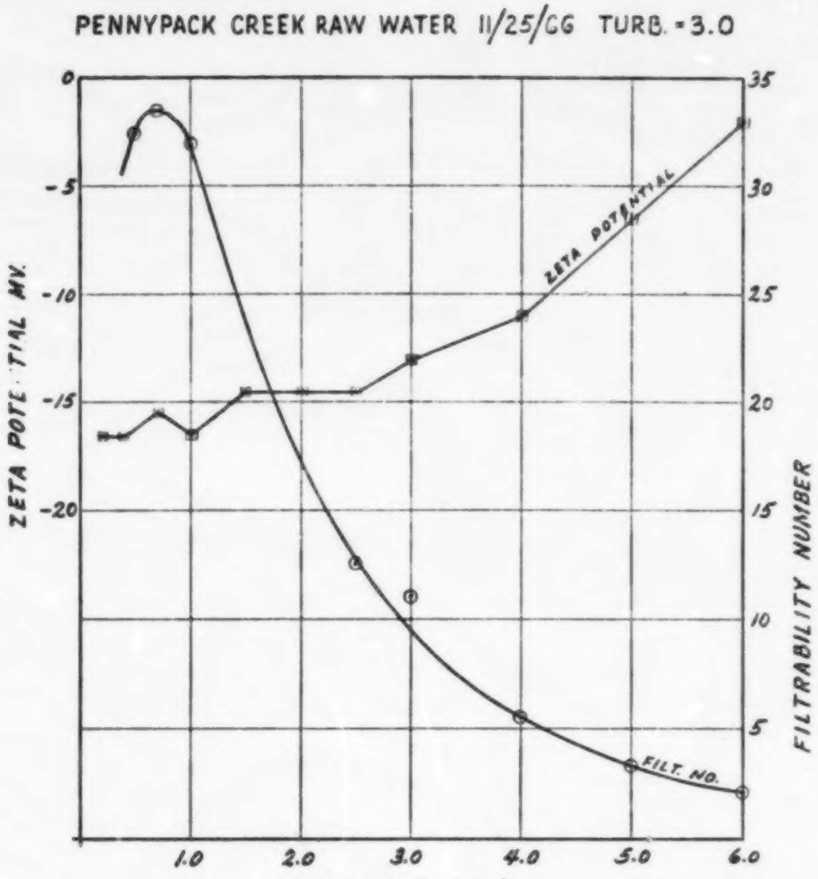
PENNYPACK CREEK RAW WATER 11/25/66 TURB. = 3.0
ZETA POTENTIAL MV.
0
-5
-10
-15
-20
FILTRABILITY NUMBER
35
30
25
20
15
10
5
ZETA POTENTIAL
FILT. NO.
1.0
2.0
3.0
4.0
5.0
6.0
ALUM DOSE - GR./GAL.

FIGURE 8

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This outline was prepared by Sidney A. Hannah, Supervisory Research Chemist, Physical & Chemical Treatment Research Program, Advanced Waste Treatment Research Laboratory, Water Quality Office, EPA, Cincinnati, OH 45226.

Descriptors: Coagulation, Flocculation, Waste Treatment, Waste Water Treatment, Zeta Potential

INTERLABORATORY QUALITY CONTROL STUDIES

I DEFINITION OF INTERLABORATORY STUDY IN EPA

A It is a between-laboratory evaluation of exact physical, chemical or biological methods which yield a measurement of desired properties.

B It is not an interlaboratory evaluation of materials, reagents, or different test conditions. It is not an initial study of a method, nor a study to develop methods.

II PURPOSES OF INTERLABORATORY STUDY

A Selection of an analytical method.

B Evaluation of an analytical method.

C Evaluation of laboratory and analyst performance.

III PREPARATION FOR INTERLABORATORY STUDY

A Laboratories are in control. Between-replicate deviation will then be very small and uniform in all laboratories.

B Ruggedness testing of a method has been completed by the method developer, or by a single qualified laboratory. Effects of small changes in time, temperature, pH, reagent and sample volume measurements, etc., are known and corrected for.(1)

C All laboratories and analysts are familiar with the method to be tested. Any special equipment or reagents required are known and available., If all analysts are not acquainted with the method, a simple preliminary study is undertaken to accomplish this.(2)

IV INSTRUCTIONS FOR THE STUDY

A Exact method write-ups are provided to each analyst. The technical description of an analytical method is difficult. The language and organization must be complete, yet simple and unambiguous. These characteristics are often conflicting.

Requirements in a method may seem trivial. Yet if these are not recognized and controlled, the entire study can be useless.

B Explicit directions for sample preservation, sample make-up, time limitations, sequence of analyses, etc., are provided.

C Advance notice of tests is given so that laboratories can integrate the test into their program and realistic deadlines can be established. Once established, deadlines for agreement to participate, for completion of analyses and for reporting are followed closely.

V THE TEST SAMPLES

A The sample is carefully designed to reflect the concentrations found in natural waters and yet be within the best portion of the concentration range for the method.

B Since precision of almost all methods varies with concentration, a comprehensive study includes several levels of concentration.

C Since accuracy of a method is very important, exactly known levels of constituents are added both to distilled and natural waters for testing. Stabilized natural waters are not used as samples.

VI SAMPLE FORM AND CONTAINER

A In EPA, Research and Monitoring Office, samples are prepared as concentrates for final dilution at the testing laboratory. Use of concentrates has several advantages. This greatly reduces space requirements and thereby greatly reduces cost of mailing. With use of sealed glass ampules, preservation of concentrates is easily obtained through steam sterilization. Chemical preservatives can be used at fairly high levels in the concentrate and can be removed as interferences by the dilution of the concentrate to final sample volume. Theoretically, a well designed sample properly sealed in glass is stable indefinitely.

B Samples can be prepared as dilute simulated or natural water samples to be analyzed, as is. These samples have an advantage in that they reach the analyst exactly as a routine sample. There is no error in making a dilution and this source of variance in the method is removed.

Some disadvantages of such samples are: the serious logistics problem from storage if a large number of samples is involved; a relatively high cost of mailing; and a limited choice of preservation methods because of the size of containers, the probable use of plastic containers, and only limited testing of water types.

VII INTERLABORATORY STUDY DESIGN

A Sample concentrates are prepared in similar yet different concentrations at each of several levels of analysis.

B True values are calculated from the amount of a constituent added, and are used to measure the accuracy of the study method.

C Multiple constituents are prepared in a sample by related groups of analyses. For example, a mineral and physical sample is analyzed for pH, alkalinity/acidity, specific conductance, total hardness, sulfate, chloride, total solids, calcium, magnesium, sodium and potassium. A nutrient sample is analyzed for ammonia nitrogen, nitrate nitrogen, Kjeldahl nitrogen, orthophosphate and total phosphorus.

D The analyst dilutes separate aliquots to volume with distilled water and with natural water of his choice.

E Single analyses are made for each parameter. Replicates are of limited value in evaluating a method for routine analyses because of data handling problems and failure to detect significant differences by replication.

F Recoveries are compared for distilled and for natural waters. Recovery from distilled water should indicate method bias. Differences in recoveries from distilled and natural waters should indicate interference by natural water samples.

VIII DATA EVALUATION AND REPORTING

A Rejection of Outliers-Accuracy.

The T-Test is applied to all data using the standard deviation of all data and compared with a T value at the 99% confidence interval. Accuracy is calculated after rejection of this data.

B Precision Measures

A statistical summary is developed for computerized treatment of data as suggested by Larsen(3). Method precision is reported as standard deviation, 95% confidence interval, and coefficient of variation. Method precision is reported for each sample since it varies with concentration.

C Graphic Display

Methods are evaluated using two-sample charts described by Youden[4]. The values reported by an analyst for a sample pair became the x and y coordinates for a single data point on the chart. Data form ellipses around the true value because of systematic error. Random error is shown as the perpendicular distance from the 45° slope. General scatter indicates poor method precision. Bias is shown by movement of the ellipse away from the true values. Outliers are visible at some distance from the major grouping of data. Examples shown on the following pages are:

Figure 1 - Outliers,
Figure 2 - Limited precision and limited accuracy,
Figure 3 - Negative bias,
Figure 4 - Positive bias,
Figure 5 - Systematic error,
Figure 6 - Good precision and accuracy.

IX INTERLABORATORY STUDY REPORT (5, 6)

A Reports are slanted toward audience.

B All data are coded by laboratory and analyst.

C Glossary of terms is necessary.

D Computer use increases statistical capabilities tremendously. There is a danger of "over-evaluation".

E Increased efficiency in reporting data is achieved by using the computer print-out directly in a report. See Figures 7 and 8.

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This outline was prepared by J. A. Winter, Chief, Method & Performance Evaluation, Analytical Quality Control Laboratory, National Environmental Research Center, EPA, Cincinnati, Ohio.

Descriptors: Laboratory Tests, Quality Control, Statistical Methods, Testing Testing Procedures

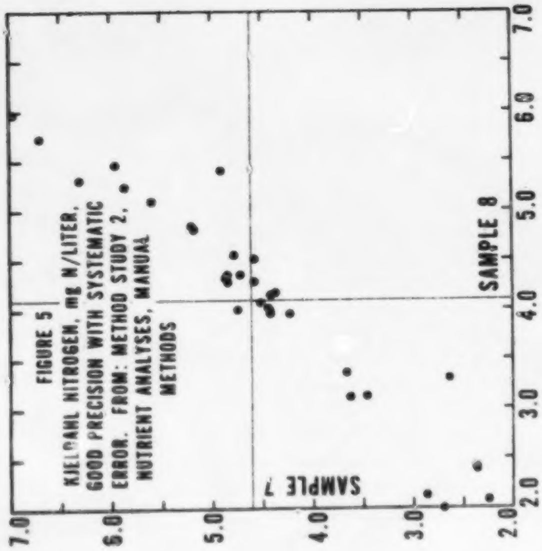


FIGURE 5
KJELDAHL NITROGEN, mg N/LITER. GOOD PRECISION WITH SYSTEMATIC ERROR. FROM: METHOD STUDY 2, NUTRIENT ANALYSES, MANUAL METHODS

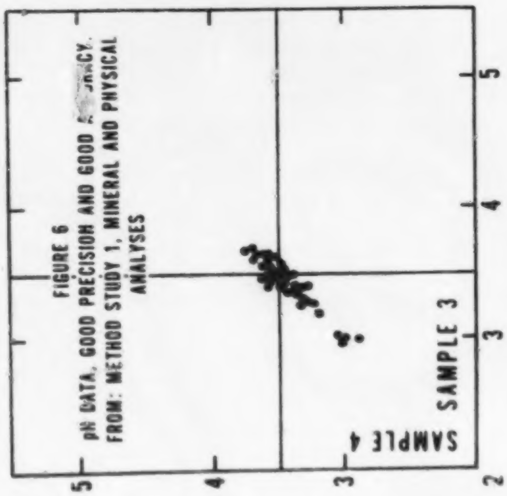


FIGURE 6
pH DATA, GOOD PRECISION AND GOOD A[illegible]CY. FROM: METHOD STUDY 1, MINERAL AND PHYSICAL ANALYSES

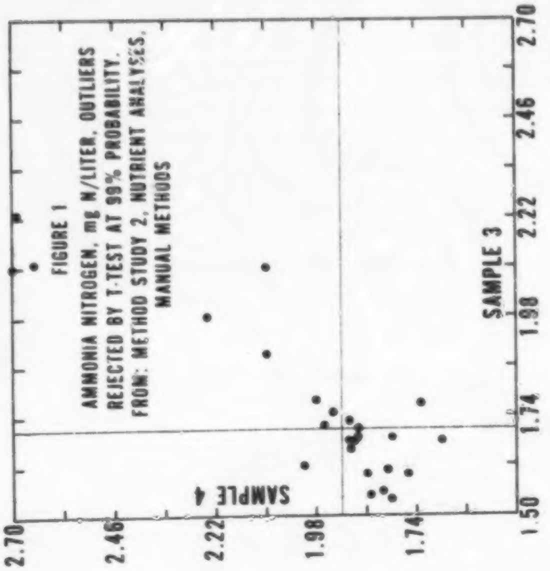


FIGURE 1
AMMONIA NITROGEN, mg N/LITER, OUTLIERS REJECTED BY T-TEST AT 99% PROBABILITY. FROM: METHOD STUDY 2, NUTRIENT ANALYSES, MANUAL METHODS

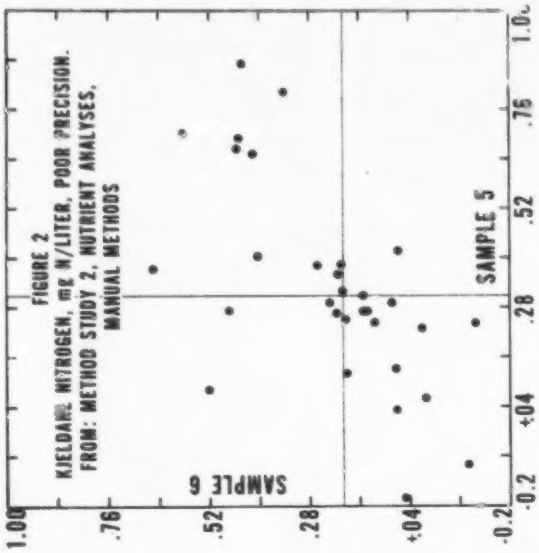


FIGURE 2
KJELDAHL NITROGEN, mg N/LITER, POOR PRECISION. FROM: METHOD STUDY 2, NUTRIENT ANALYSES, MANUAL METHODS

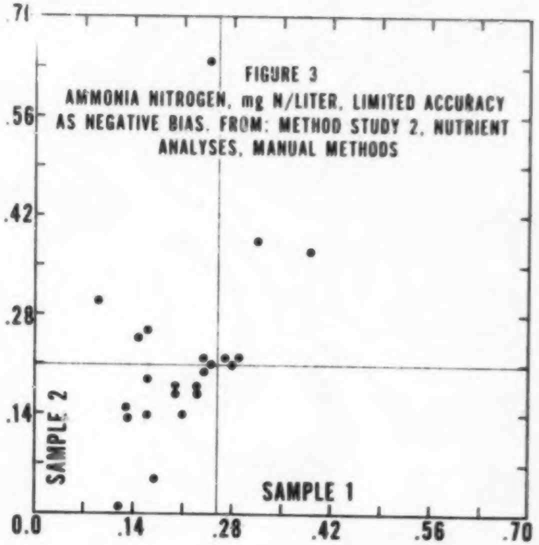


FIGURE 3
AMMONIA NITROGEN, mg N/LITER, LIMITED ACCURACY AS NEGATIVE BIAS. FROM: METHOD STUDY 2, NUTRIENT ANALYSES, MANUAL METHODS

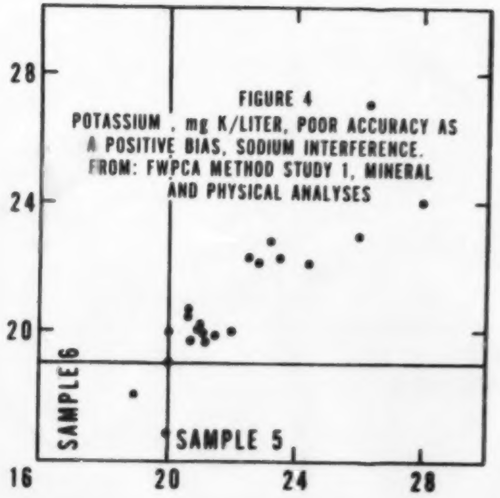


FIGURE 4
POTASSIUM , mg K/LITER, POOR ACCURACY AS A POSITIVE BIAS, SODIUM INTERFERENCE. FROM: FWPCA METHOD STUDY 1, MINERAL AND PHYSICAL ANALYSES

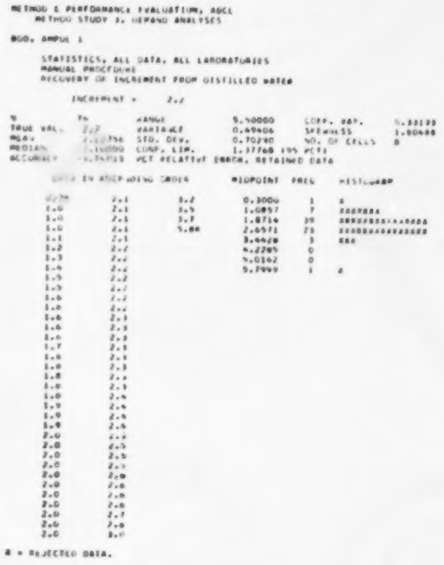
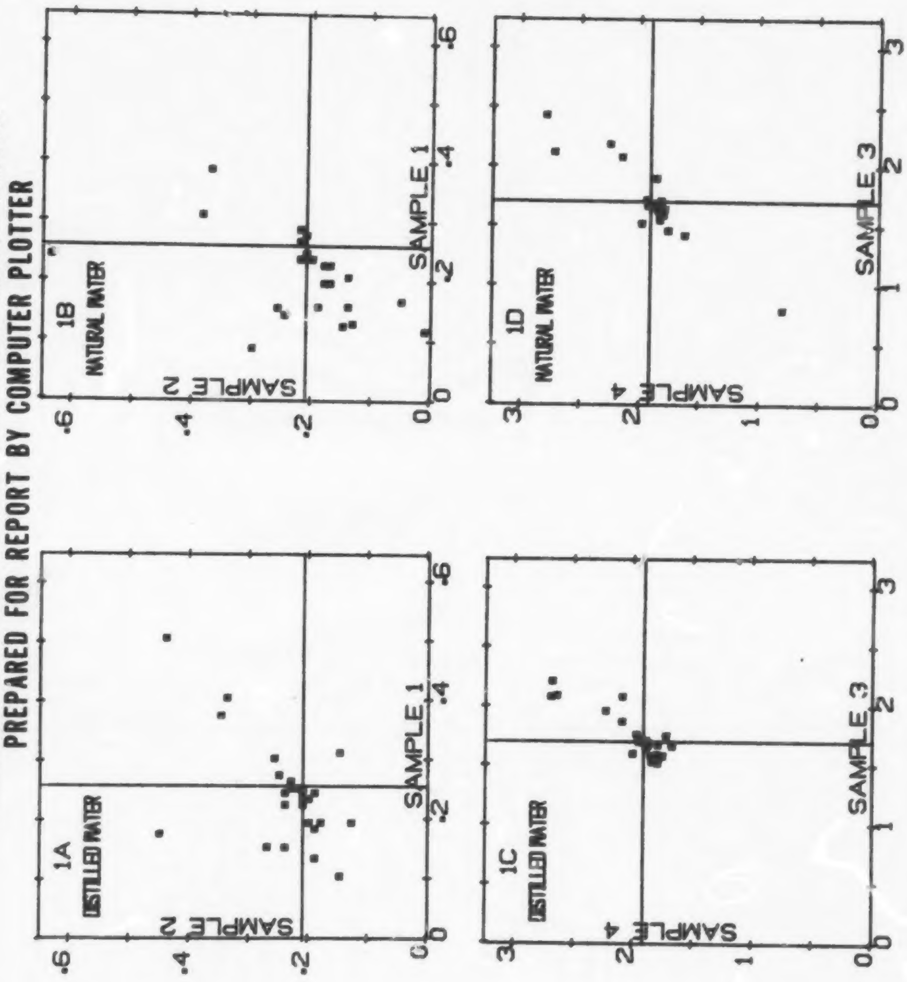


FIGURE 7
BOD DATA, STATISTICAL SUMMARY. PREPARED BY COMPUTER FOR DIRECT USE IN REPORT.
FROM: METHOD RESEARCH STUDY 3, DEMAND ANALYSES

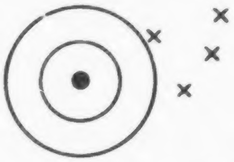
FIGURE 8
AMMONIA NITROGEN DATA, YOUDEN'S TWO-SAMPLE CHARTS.
PREPARED FOR REPORT BY COMPUTER PLOTTER



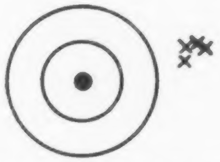
ACCURACY-PRECISION-ERROR

I INTRODUCTION

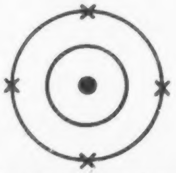
An analytical method is subject to errors. These errors may affect the accuracy of the method because they introduce bias into the results. There are other types of errors which affect the precision of the method because they produce random fluctuations in the data. The most desirable situation for the analyst is shown in the diagram in Figure 1 where the results are both precise and accurate.



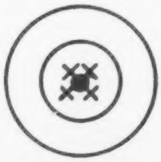
IMPRECISE AND INACCURATE



PRECISE BUT INACCURATE



ACCURATE BUT IMPRECISE



PRECISE AND ACCURATE

Figure 1. PRECISION AND ACCURACY

A Accuracy

For results to be accurate, the analysis used must give values close to the true value.(See Figure 1)

B Precision

Precision is the degree of agreement among results obtained by repeated measurements on a single sample under a given set of conditions. It is a measure of the degree to which results "check." (See Figure 1)

C Note

It is possible to have precision without accuracy. (See Figure 1)

II DETERMINATE ERROR AND ACCURACY

A determinate error is one which contributes a constant error or bias to results, causing them to be inaccurate. This constant error makes it possible for results to agree with each other (be precise) and still be inaccurate.(See Figure 1)

Determinate errors have "assignable" causes which can usually be identified and either eliminated or controlled. (The terms "determinate" error, "assignable" error, and "systematic" error are synonymous).

A Sources of Determinate Error

1 Method error

Method errors are those that are inherent in the procedure. These are very serious and the hardest to detect and correct. The most common method error is the presence of interferences in the sample. Other examples would be precipitation of substances other than the desired material, partial solubility of precipitates, and entrainment as in a solvent extraction procedure.

2 Personal errors

Personal errors are attributable to individual mistakes which are consistently made by an analyst. These errors are the result of consistent carelessness, lack of knowledge or personal bias. Examples are errors in calculations, use of contaminated reagents, non-representative sampling, or poor calibration of standards and instruments.

3 Instrumental errors

Instrumental errors are those which are caused by an analytical instrument or by the effects of the environment acting on the instrument. Moisture in a G.C. column, improper wavelength markings on a spectrophotometer or incorrect scoring on a buret would be examples.

B Effects of Determinate Error

1 Additive

An additive determinate error is one which has a constant value regardless of the amount of analytically sought constituent present in the sample. (See Figure 2)

2 Proportional

A proportional determinate error changes value according to the amount of analytically sought constituent in the sample. (See Figure 3)

III DETECTION OF DETERMINATE ERROR

A Spiked Samples

1 Samples which can be determined before and after the addition of a known constituent (in the concentration range of interest) provide a way to detect determinate errors. Spiked samples should be representative and resemble actual conditions as closely as possible. The quantitation of bias can then be obtained with the following measures.

a Mean error - the difference between the mean of the data and the true result.

b Relative error - the mean error of a set of data expressed as a percentage of the true result.

EXAMPLE: An analyst determines the nitrate content of the effluent from his sewage treatment plant to be 0.5 mg/l. He then adds 1 mg/l of standard nitrate solution to the sample. Table 1 shows the replicate results obtained on the spiked sample, and calculation of both mean and relative errors.

Table 1

Sample: Effluent
Determination: Nitrate (Modified Brucine)

X_i
1.55 mg/l
1.58
1.60
1.47
1.35

1 Calculation of mean error

$$\bar{X} = \frac{7.55}{5} = 1.51 \text{ mg/l}$$

Mean error = +0.01 mg/l

2 Calculation of relative error

$$\text{Relative Error} = \frac{0.01 \times 100}{1.50} = 0.7\%$$

---

2 Control charts

Trends and shifts on control charts may also indicate determinant error. Using spiked samples, the standard deviation is calculated and control limits (usually $\pm$ 3 standard deviations) for the analysis are set (see Figure 4. For further discussion of control limits, see reference 3, p. 62.

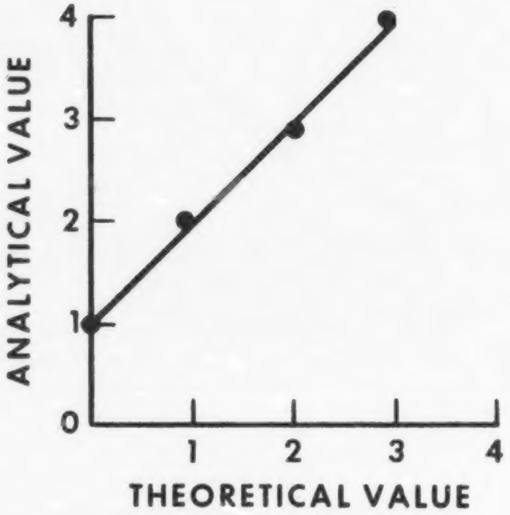


Figure 2. ADDITIVE ERROR

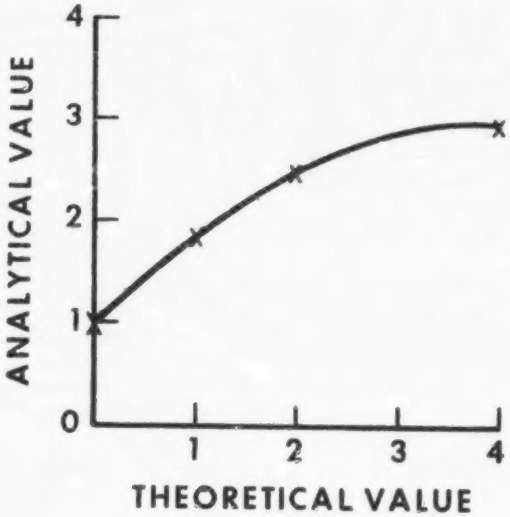


Figure 3. PROPORTIONAL ERROR

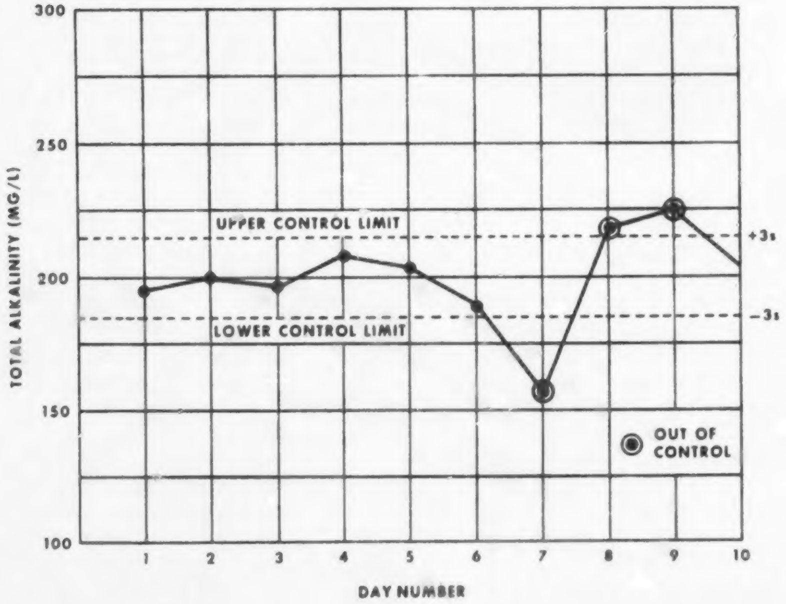


Figure 4. CONTROL CHART

3 In some cases, it is impossible to spike a sample so that it resembles actual conditions (e.g., BOD and pesticide samples). Youden (11) provides excellent techniques for detecting bias in this situation.

B Unknown Samples

1 Independent method

Analysis of a sample for a desired constituent by two or more methods that are entirely different in principle (gravimetric and volumetric) may aid in the estimation of determinate error. However, another reliable method may not be available or may be laborious to perform.

2 Control charts

It is possible to plot a control chart (Figure 4) even when it is not possible to spike a sample. One can use as a reference value an average of a series of replicate determinations performed on a composite check sample. Such a sample must be preserved or stabilized in such a way that the concentration of the constituent being measured will not change from day to day (see reference 1).

3 Aliquoting

If the determinate error is additive, the magnitude may be estimated by plotting the measured quantity versus a range of sample volumes or sample weights. If the error has a constant value regardless of the amount of analytically sought constituent, then a straight line fitted to the points will not go through the origin. (See Figure 5)

C Youden's Graphical Technique (10, 11, 12)

Dr. W. J. Youden has devised an approach to test for determinant errors with a minimum of effort on the part of the analyst and his laboratory. Samples used may be of known (spiked) or unknown composition.

1 Technique

Two different test samples (X and Y) are prepared and distributed for analysis to as many individuals or laboratories as possible. Each participant is asked to perform only one determination on each sample (NOTE: It is important that the samples be relatively similar in concentration of the constituent being measured). Table 2 shows the results on two such samples analyzed for percent potassium by 14 different laboratories. The mean for each sample has been calculated.

2 Interpretation - Figure 6

The vertical line drawn on the graph represents the mean $(\overline{X})$ of all the results obtained on Sample X; the horizontal line was drawn through the mean $(\overline{Y})$ of all the results obtained on sample Y. Each pair of laboratory results can then be plotted as a point on the graph (marked X. 1 etc.)

If the ratio of the bias (error) to standard deviation is close to zero for the determinations submitted by the participants, then one would expect the distribution of the paired values (or points) to be close to equal among the four quadrants. The fact that the majority of the points fall in the (+, +) and (-, -) quadrants indicates that the results have been influenced by some source of bias or determinate error.

Furthermore, one can even learn something about a participant's precision from the graph. If all participants had perfect precision (no indeterminate error), then all the paired points would fall on a 45° line passing through the origin. Consequently the distance from such a 45° line of each participant's point provides an indication of that participant's precision.

3 The Youden method is also discussed in this manual in the section on Interlaboratory Quality Control Studies. A quantitative treatment of this subject can be found in references 11 and 12.

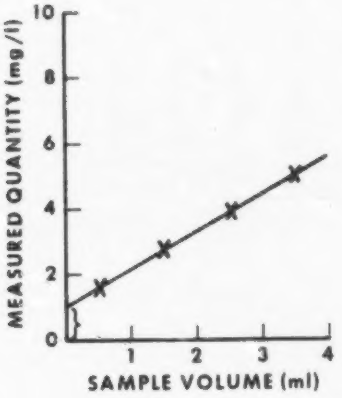


Figure 5

Table 2

Laboratory	Sample X	Sample Y
1	9.74%	8.50%
2	9.92	8.28
3	9.98	8.84
4	9.99	8.24
5	10.00	8.73
6	10.11	8.54
7	10.12	8.64
8	10.14	8.82
9	10.19	9.04
10	10.23	8.93
11	10.25	8.97
12	10.29	8.80
13	10.55	9.21
14	10.62	8.95

$\overline{X} = 10.15$ $\overline{Y} = 8.75$

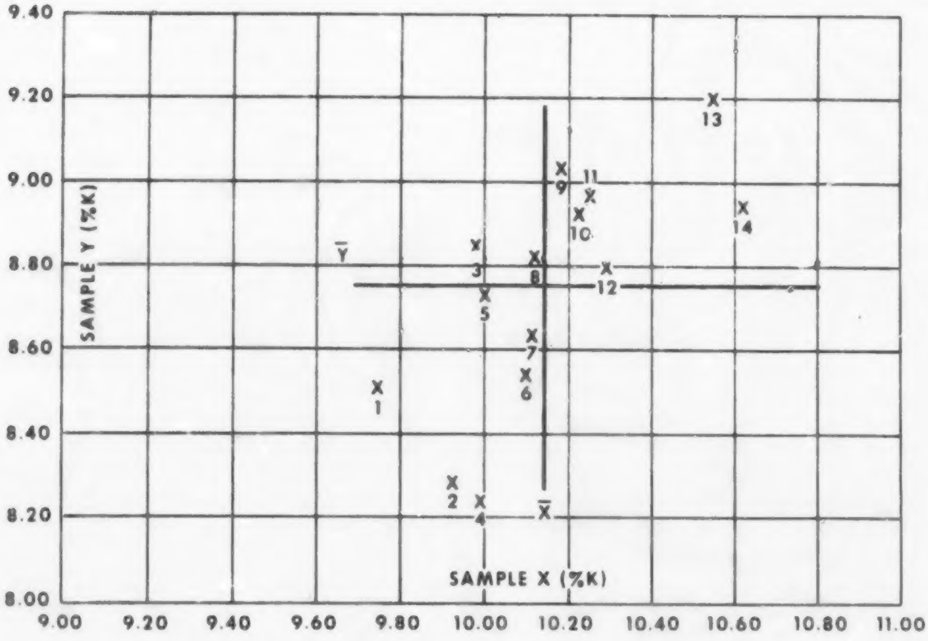


Figure 6. YOUDEN'S GRAPHICAL TECHNIQUE

IV ELIMINATION OF DETERMINATE ERROR

Various approaches can be used to eliminate the source of determinate error. The approach used depends upon whether the source is personal, method, or instrumental.

A Personal

Great care must be used to avoid producing an unconfident attitude in a technician. It is undesirable for the analyst to feel he is being "policed."

B Method and Instrumental

1 Blanks

Blanks can be used to correct for interferences from reagents, sample color, etc.

2 Correction factors

Examples of correction factors used in environmental analyses to eliminate determinate errors are the following:

a Recovery factors in organic extractions

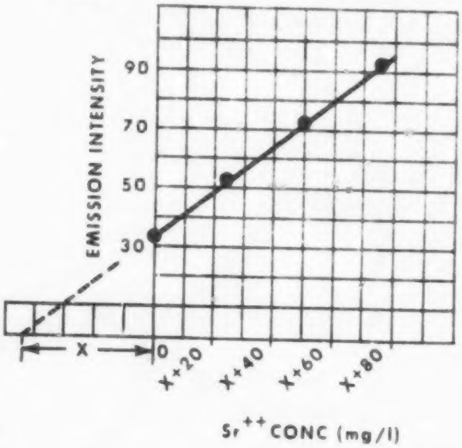
b Chemical yield values in gravimetric analyses

c Counting efficiencies for radiation counters

3 Standard addition

Sample interferences producing determinate errors can be overcome by adding equal amounts of unknown sample to a series of standards. The concentration of the unknown can then be determined graphically from a plot of the measured quantity (absorption, emission, etc.) versus the standard concentration. (See Figure 7)

Figure 7. METHOD OF STANDARD ADDITION GRAPHICAL METHOD OF COMPUTING Sr CONCENTRATION



NOTE: The X value appearing along the abscissa (i.e., X + 20, X + 40, etc.) refers to the unknown added to the strontium standard

4 Standard compensation

Another approach is to prepare the standard so that its composition resembles that of the sample as closely as possible. The objective of the approach is exactly the same as that of standard addition - to compensate for the presence of interfering substances in the unknown.

V INDETERMINATE ERROR AND PRECISION

Even when all determinate errors are eliminated, every replicate analysis will not give the same value. Such variation in results is due to indeterminate error, also known as random, chance or uncontrollable error. Indeterminate error affects the precision or agreement among results.

A Sources of Indeterminate Error

Indeterminate errors are due to unassignable or "chance causes." Examples are inadvertent contamination of sample or glassware, variation in reagent additions, or variations in instrument response (see Figure 8).

B Effects of Indeterminate Error

Since the causes of these errors are random, the effects are also random. Fortunately, these random variations conform to the "Laws of Chance" so statistical measures of precision can be used to quantitate indeterminate errors.

VI DETECTION OF INDETERMINATE ERROR

A measure of the degree of agreement among results can be obtained by analyzing a single sample repeatedly under a given set of conditions.

A Range

The range of the replicate results (difference between the lowest and the highest value) provides a measure of indeterminate variations.

B Standard Deviation

An estimation of indeterminate error can be obtained through a calculation of the standard deviation. The following formulas should be applied to random data which follows a normal distribution. Normality can be checked by ranking and plotting the data on normal probability paper; it should fall on a straight line (see Figure 9). Any values which do not fall close to the straight line (those values encircled) should be rejected in the calculation of standard deviation. Other statistical tests (8) can be used to objectively evaluate the rejection of outliers. The value obtained for standard deviation for a particular method may vary with the analyst, the concentration range of the constituent, and the composition of the sample analyzed. The confidence of the estimate is increased as the number of results (n) used to compute the standard deviation is increased.

1 Replicate results on the same sample:

$$s = \sqrt{\frac{\Sigma(X_i - \overline{X})^2}{n-1}} \qquad (1)$$

X_i = value of single result

$\overline{X}$ = average (mean) of results on same sample

n = number of results

Example: Table 3 contains a set of 5-day BOD results obtained on a synthetic sample containing 150 mg/l of glucose and 150 mg/l glutamic acid. (Note: A 1% dilution was used in the actual test). The results are those submitted by laboratories participating in a colaborative study. Calculate the standard deviation of these results.

$\overline{X}$ = 192 mg/l

n = 36.0

$\Sigma(X_i - \overline{X})^2$ = 58,200

$$s = \sqrt{\frac{58,200}{35}}$$

s = 41 mg/l

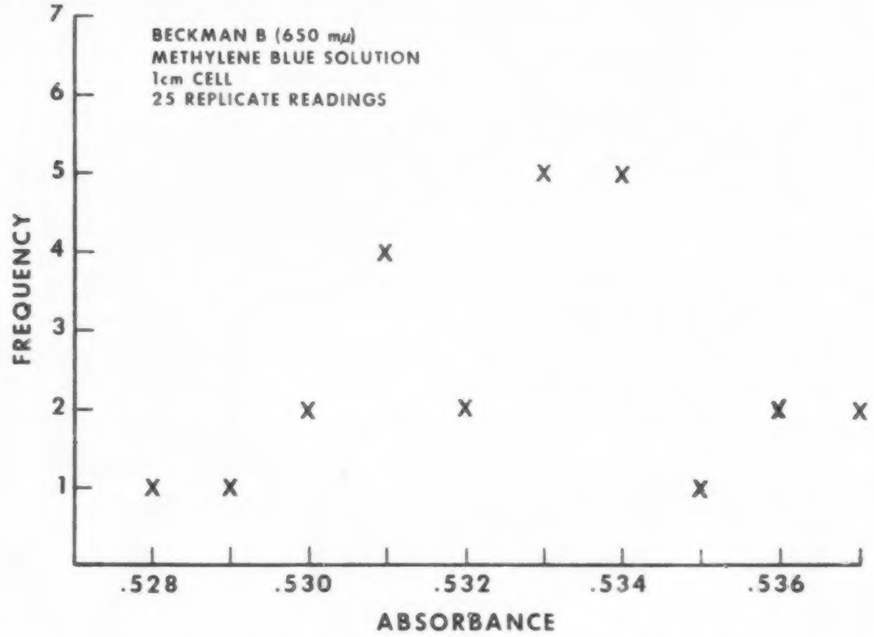


Figure 8. VARIATIONS IN SPECTROPHOTOMETER READINGS

FIGURE 9 NORMAL PROBABILITY CURVE

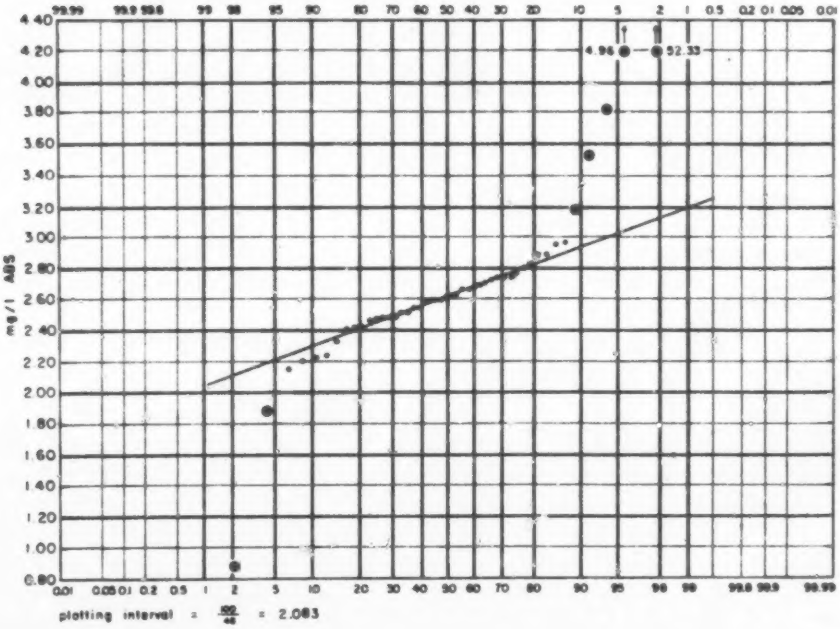


Table 3

SAMPLE: 150 mg/1 glucose + 150 mg/1 glutamic acid (1% dilution)
DETERMINATION: 5-day Biochemical Oxygen Demand

X_i	$X_i - \bar{X}$	$(X_i - \bar{X})^2$	X_i	$X_i - \bar{X}$	$(X_i - \bar{X})^2$
100 mg/1	-92 mg/1	8464 $(mg/1)^2$	198 mg/1	6 mg/1	36 $(mg/1)^2$
117	-75	5625	199	7	49
125	-67	4489	200	8	64
132	-60	3600	200	8	64
142	-50	2500	204	12	144
147	-45	2025	210	18	326
153	-39	1521	211	19	361
160	-32	1024	212	20	400
165	-27	729	215	23	529
165	-27	729	223	31	961
167	-25	625	224	32	1024
173	-19	361	227	35	1225
178	-14	196	229	37	1369
189	- 3	9	238	46	2116
190	- 2	4	247	55	3025
196	4	16	250	58	3344
196	4	16	259	67	4489
197	5	25	274	82	6724

*Data taken from Water, Oxygen Demand Report (July, 1960), Analytical Reference Service, Training Program, R.A. Taft Sanitary Engineering Center, Cincinnati, Ohio,

Table 4*

SAMPLE: Aqueous
DETERMINATION: Phosphate (Lucerna, Conde, and Prat Method)
CONCENTRATION RANGE: 30 - 70 mg/1

Sample	Mg/1 P	d	d^2	Sample	Mg/1 P	d	d^2
A	51 53	2	4	L	54 56	2	4
B	39 39	0	0	M	38 37	1	1
C	53 54	1	1	N	52 52	0	0
D	47 47	0	0	O	58 58	0	0
E	50 51	1	1	P	54 54	0	0
F	48 47	1	1	Q	54 54	0	0
G	50 50	0	0	R	48 48	0	0
H	47 47	0	0	S	52 51	1	1
I	42 42	0	0	T	53 53	0	0
J	50 51	1	1	U	46 47	1	1
K	59 60	1	1	V	42 42	0	0

*Data obtained from Frank Schickner, Proctor and Gamble Company.

2 Duplicate results on different samples

In a laboratory where duplicates are routinely run, it would be simple to use the following formula for evaluation of standard deviation.

$$s = \sqrt{\frac{\Sigma(d^2)}{2k}} \quad \text{(See reference 5, page 654)} \qquad (2)$$

d = difference between duplicates

k = number of samples

Example: Table 4 contains a set of phosphate results obtained on aqueous samples in the range 100-200 mg/l. Calculate the standard deviation of these results.

$\Sigma(d^2) = 16$

$k = 22$

$s = \sqrt{\frac{16}{44}}$

$s = .61$ mg/l

3 Duplicate and triplicate results on different samples

$$s = \sqrt{\frac{\Sigma(X_i - \bar{X})^2}{n - k}} \quad \text{(See reference 7, page 73)} \qquad (3)$$

Note: $\bar{X}$ = average of results on the same sample

Example: Table 5 contains a set of % nitrogen results obtained on unknown organic compounds.

Calculate the standard deviation.

$\Sigma(X_i - \bar{X})^2 = .2401$

$n - k = 22$

$s = \sqrt{\frac{.2401}{22}}$

$s = .104\%$

4 Use of range to estimate standard deviation

For a small number of replicates ($n < 10$), the range can be used to estimate the standard deviation (See Table 6).

$$\frac{R}{d_N} = s \qquad (4)$$

Example: Table 7 contains a set of replicate nitrate results. Calculate the standard deviation of these results.

a Use of formula (1)

$\bar{X} = 0.72$ mg/l

$n = 5$

$\Sigma(X_i - \bar{X})^2 = .0134$

$s = \sqrt{\frac{.0134}{4}}$

$s = .058$ mg/l

b Use of formula (4)

$R = .14$ mg/l

$d_N = 2.33$

$s = \frac{.14 \text{ mg/l}}{2.33}$

$s = .060$ mg/l

C Coefficient of Variation

An estimation of indeterminate error can also be made by calculating the coefficient of variation.

$$V = \frac{s}{\bar{X}} \times 100$$

By comparing the standard deviation (dispersion) to the average or mean value (central tendency) in a set of data and expressing this relative standard deviation as a percentage, the analyst has a meaningful interpretation of the degree of dispersion present. Indeter-

Table 5*

SAMPLE: Unknown Organic Compounds
DETERMINATION: % Nitrogen (Kjeldahl)

CONCENTRATION
RANGE: 10% - 20%

Sample	%N	$\overline{X}$	$X_i - \overline{X}$	$(X_i - \overline{X})^2$	Sample	%N	$\overline{X}$	$X_i - \overline{X}$	$(X_i - \overline{X})^2$
A	16.48	16.47	.01	.0001	J	13.93	13.75	.18	.0324
	16.46		.01	.0001		13.56		.19	.0361
B	16.50	16.50	0	0	K	10.34	10.27	.07	.0049
	16.49		.01	.0001		10.19		.08	.0064
C	16.72	16.65	.07	.0049	L	17.16	17.15	.01	.0001
	16.57		.08	.0064		17.13		.02	.0004
D	17.52	17.58	.06	.0036	M	15.01	15.03	.02	.0004
	17.60		.02	.0004		15.05		.02	.0004
	17.63		.05	.0025					
E	16.31	16.31	0	0	N	12.44	12.62	.18	.0324
	16.30		.01	.0001		12.70		.08	.0064
						12.73		.11	.0121
F	16.40	16.35	.05	.0025	O	14.37	14.37	0	0
	16.31		.04	.0016		14.36		.01	.0001
	16.35		0	0					
					P	11.85	11.85	0	0
G	17.56	17.55	.01	.0001		11.85		0	0
	17.54		.01	.0001	Q	14.79	14.73	.06	.0036
H	14.96	14.81	.15	.0225		14.70		.03	.0009
	14.66		.15	.0225		14.70		.03	.0009
					R	17.19	17.17	.02	.0004
I	19.15	19.02	.13	.0169		17.14		.03	.0009
	18.89		.13	.0169					

*Data obtained from Frank Schickner, Proctor and Gamble Company.

Table 6*

FACTORS USED TO ESTIMATE THE STANDARD DEVIATION FROM RANGE

Size of Sample (n)	d_N	$\frac{1}{d_N}$
2	1.13	.887
3	1.69	.591
4	2.06	.486
5	2.33	.430
6	2.53	.395
7	2.70	.370
8	2.85	.351
9	2.97	.337
10	3.08	.325

*Natrella, Experimental Statistics,(8) pp. 2-6.

Table 7

SAMPLE: Ohio River Water

DETERMINATION: Nitrate (Modified Brucine)

X_i	$X_i-\overline{X}$	$(X_i-\overline{X})^2$
0.65 mg/lN	-0.07	.0049
0.68	-0.04	.0016
0.70	-0.02	.0004
0.76	+0.04	.0016
0.79	+0.07	.0049

minate error wouldbe the cause of undue dispersion.

Example: Table 3

$s = 41$ mg/l

$\overline{X} = 192$ mg/l

$V = \frac{41}{192} \times 100$

$V = 21\%$

D An indication of an individual's analytical precision can be obtained by his participation in an interlaboratory study. In this case, the analyst performs only one determination on each of two samples. Using Youden's Graphical Technique as an indication of an individual's precision was discussed previously in this outline in III C. Also see the outline on "Interlaboratory Quality Control Studies."

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This outline was prepared by Betty A. Punghorst, Chemist, formerly with National Training Center, and revised by Audrey E. Donahue, Chemist, National Training Center, MDS, WPO, EPA, Cincinnati, Ohio 45268

Descriptors: Data Collections, Data Processing, Measurement, Monitoring, Quality Control

A QUICK LOOK AT STATISTICS

I INTRODUCTION

Quantitative statistical analysis has been publicized by some analyst as a cure all. This exaggerated claim is adhered to by only a few opportunists.

Quantitative analysis should be thought of as a valuable tool which can be expoited by close cooperation between the statistician and the investigator in a joint attempt to find better methods of controlling or understanding the complex interaction of men, materials, machines and the natural environmental resources.

Modern statistics is still a relatively new subject with a long list of unsolved statistical problems. Unless caution is observed the inevitable result will be some unhappy experiences with inadequately equipped amateurs. In point of fact, most statistical procedures are quite simple and intuitively acceptable once they have been pointed out in a particular context. Heuristic persuasion can be effectively used to give insights into the concept, development and foundations of the particular tool used.

II IMPORTANCE OF PREPLANNING

Many investigators now believe that statistics and particularly the statistical design of data collection has potentially impressive leverage effects on the amount of information to be included and obtainable from any research effort.

After all, any finite body of data contains only a limited amount of information. This limit is set by the inherent nature of the data themselves and cannot be increased by any amount of ingenuity exercised by the data analyst. However intelligent preplanning can increase this limit.

Statistical methods cannot reveal anything that is not already implicit in the data. However, every correct inference of interest possible should be made. A continual effort should be made to recover all of the information but there never will be a way to find more than is already there.

It would be better to do enough work on five projects than an inadequate amount on a much larger number. You don't have to plan in order to fail, all you have to do is fail to plan.

It is well known that the end doesn't justify the means but in data collection and analysis the end dictates the means. The importance of preplanning cannot be overemphasized. If you fail to plan then you are planning to fail.

Many investigators still believe that statisticians only enter at the end of the work--that is, at the final examination of the data. It is small comfort to the investigator to be told after the event, that the answers to his questions are inconclusive and were bound to be so because an inadequate amount of effort was allotted to the work.

When anyone has a heart attack there is no doubt or hesitation about who should be consulted. By analogy data collection, analysis and interpretation usually benefits with the cooperation of a statistician.

The only real barrier to consultation seems to be the excessive pride of some scientists who do not want to admit that they are not experts in two different fields. In this day and age due to the tremendous increase in scientific knowledge, we no longer have experts in one field but only specialists whose expertise narrows as time progresses.

When people talk to a specialist after they have a heart attack, nothing is held back. There is free access to all information. In the same way when consulting with a statistician, lay all the cards on the table. Even if one is not sure about the relevance of a topic, it should be brought up. The multi-discipline interaction during the application of the scientific method will determine a factor's contribution or influence on the problem.

The definition of the scientific method that I like best is "The scientific method, as far as it is a method, is nothing more than doing one's damnedest with one's mind, no holds barred", reference 1.

This excellent definition provides no recommended program of action to follow. Any scientific investigation can be viewed as a five step process. These are:

1 Problem definition
2 Data collection scheme
3 Actual data collection
4 Data analysis
5 Written report or recommendations

The most important step is the first one because the second is based upon it. The third follows from the second, etc. The process is like a chain with five links. If the first link is incorrect, the entire chain is. Too often, too little time is spent on step one. Many times the problem is vaguely or ill defined. The best protection against these faults is to write down black on white a statement of the problem and have all connected with the investigation to agree on the statement. With this as an introduction we proceed to describe the field of statistics briefly.

FIGURE 1

FOURFOLD MAP OF ENDS AND MEANS IN APPLIED STATISTICS

				Source Or Means Of Data Acquisition	
				Nonexperimental	Experimental
PURPOSE OR ENDS OF STATISTICS	Enumerative or Descriptive	Answers: question, how much	Aim: adjustment to the environment	Tables and graphs Averages central tendency Dispersion measures Frequency curves Correlation Survey sampling Index numbers	Same Assessment of probabilities Theoretical means, variances,etc. and corresponding confidence intervals
	Explanatory or analytic causal inference	Answers: question, why	Aim: ascendancy over the environment	Specialized branches of applied statistics Demography Econometrics Genetics Causal inference from time series	Design of factorial experiments Randomization methods Significance tests Regression analysis Analysis of variance and covariance Probit analysis

III ALTERNATIVE VIEWS OF STATISTICS

The field of statistics can be described from many different angles. Each viewpoint results in a different insight into this vast discipline. In this introduction we consider the entire field from three viewpoints. The first view is very simple, the second introduces more details and finally the last viewpoint is very comprehensive with respect to subject matter but is only one molecule deep.

IV FIRST VIEWPOINT

The first way of looking at statistics is to partition the data source into the method of acquisition as either experimental or nonexperimental.

In an experimental investigation, control is exercised over some or all of the influencing variables, while in a nonexperimental investigation nature is merely measured as is without any control on any of the influencing variables.

The purpose of the analysis is also viewed as two-fold, that is, either enumerative or analytic. The conbination of double means and ends leads to the four-fold table in Figure 1. This is taken essentially from reference 2.

Historically, the field of statistics started with the upper left quadrant about the turn of the century. The research proceeded clockwise until the lower left hand quadrant was reached. That is causal inference from observational data. This quadrant is the most difficult and has been receiving the most research attention lately.

In each quadrant some of the techniques are indicated and the reader should be further cautioned that the lines drawn are fluid and are not rigid. A partial listing has been given in each quadrant and no attempt has been made to give a 100% enumeration.

V SECOND VIEWPOINT

The next viewpoint of statistics is in this orientation the one taken by The International Statistical Institute. Figure 2 shows their scheme for classification of abstracts. Any article in statistics can be classified in one or more of the indicated titles. Some articles are given a primary classification and others in addition are given a secondary classification.

Let us give a brief introduction into the major topics listed in Figure 2.

Upon inspection of the subtitles of the first topic, mathematical methods, it is evident that these subtitles are a list of the mathematical techniques used by the professionals to solve the difficult problems encountered in the development of the theory and methods.

These are the tools used to extend the present boundaries of knowledge. They are not statistics per se but are an indispensable aid to the research statistician.

The next topic, probability, can be described as a method for quantitizing uncertainty where the limits of uncertainty are a numerical value which does not lie outside the range from zero to one. Most people have a correct intuitive feel for probability. If an event can happen (i.e., it will rain) say with probability 0.9 (or 90%) then one knows that the event could or could not occur. However, for a large number of trials, on the average nine times out of ten it will occur (or rain) and one in ten it won't.

FIGURE 2

SCHEME FOR CLASSIFICATION OF ABSTRACTS

0. MATHEMATICAL METHODS (White)
   0. General papers
   1. Solution of equations
   2. Methods of curve and surface fitting; smoothing
   3. Interpolation and quadrature
   4. Special functions and transforms
   5. Functional relationships
   6. Determinantal and matrix analysis
   7. Game theory
   8. Programming techniques
   9. Group and field theory
   10. Graph theory and combinatorial analysis
   11. Measure theory
   12. Optimisation

1. PROBABILITY (Pink)
   0. General papers
   1. Calculus of probabilities
   2. Expected values
   3. Combinatorial problems
   4. Geometric probability
   5. Limit theorems
   6. Stochastic convergence
   7. Stochastic approximation
   8. Decision theory and functions
   9. Transforms: Fourier, Laplace, etc.
   10. Convolutions

2. FREQUENCY DISTRIBUTIONS (Green)
   0. General papers
   1. Descriptive properties
   2. Transformations of variates
   3. Normal and lognormal
   4. Binomial, multinomial and hypergeometric
   5. Poisson, expoential, negative binomial, logarithmic and contagious
   6. Rectangular, extreme value and Weibull
   7. Pearson and "series expansion" distributions
   8. Truncated and mixed distributions
   9. Multivariate distributions
   10. Limit distributions
   11. Approximations
   12. Other distributions

3. SAMPLING DISTRIBUTIONS (Light Blue)
   0. General papers
   1. t, z, F and x^2 distributions
   2. Non-central distributions
   3. Studentisation
   4. Quadratic forms
   5. Correlation and regression coefficients
   6. Location and scale statistics
   7. Shape and other descriptive statistics
   8. Order statistics
   9. Multivariate problems
   10. Limit distributions
   11. Linear forms

4. ESTIMATION (Yellow)
   0. General papers
   1. Properties of estimators
   2. Types of estimator: Bayes, maximum likelihood, least squares, etc.
   3. Individual estimators: point
   4. Individual estimators: interval
   5. Inequalities: tolerance limits and regions
   6. Distribution-free methods
   7. Sequential methods
   8. Multivariate problems
   9. Finite population procedures - surveys
   10. Simultaneous estimation
   11. Distribution functions and densities
   12. Decision theory

5. HYPOTHESIS TESTING (Purple)
   0. General papers
   1. Properties of test
   2. Individual hypotheses
   3. Two-sample problem
   4. k-sample problem
   5. Outliers
   6. Distribution-free tests
   7. Sequential tests
   8. Multivariate problems
   9. Types of test: likelihood ratio, Bayes, minimax, etc.
   10. Goodness-of-fit tests
   11. Combining and comparing tests
   12. Decision theory

FIGURE 2 (continued)

6. RELATIONSHIPS (Grey)
    0. General papers
    1. Regression; linear hypothesis, polynomials
    2. Correlation inc. canonical correlation
    3. Factor methods and principal components
    4. Discriminant analysis and cluster analysis
    5. Ranking and scaling methods
    6. Systems of equations: structure
    7. Non-linear equations-logistic
    8. Transformed relationships-quantal response
    9. Association and contingency
    10. Functional relationships
    11. Non-standard conditions
    12. Other multivariate methods

7. VARIANCE ANALYSIS (Biscuit)
    0. General papers
    1. Fixed effects model
    2. Variance components model
    3. Mixed and other models
    4. Non-orthogonal data and missing values
    5. Non-standard conditions-failure of assumptions
    6. Covariance analysis
    7. Multiple comparisons; multiple decision procedures
    8. Ranked data
    9. Sequential methods inc. preliminary tests
    10. Combining sets of results
    11. Precision of measurement
    12. Multivariate models

8. SAMPLING DESIGN (Orange)
    0. General papers
    1. Simple random; stratified; multi-stage
    2. Sampling with unequal probability
    3. Multi-phase sampling; double sampling
    4. Natural (human, animal and biological) populations
    5. Non-sampling problems
    6. Censored, systematic and quota sampling
    7. Nature and number of units; cost and efficiency
    8. Acceptance inspection
    9. Process control

9. DESIGN OF EXPERIMENTS (Blue)
    0. General papers
    1. Block designs; designs for two-way elimination of heterogeneity
    2. Factorial arrangements
    3. Response surfaces
    4. Nature of unit; number of replications; cost and efficiency
    5. Paired comparisons and matching problems
    6. Preference tests
    7. Repeated and sequential experiments
    8. Weighing problems
    9. Sensitivity problems
    10. Systematic designs
    11. Screening tests
    12. Other designs, e.g., mixtures

10. STOCHASTIC PROCESSES AND TIME SERIES (Red)
    0. General papers
    1. Properties of individual process
    2. Estimation problems
    3. Tests of hypotheses
    4. Queueing, storage, risk and congestion theory
    5. Information theory
    6. Stationary processes and spectral analysis
    7. Auto and serial correlation
    8. Multivariate processes
    9. Biological population studies; genetic models
    10. Renewal theory
    11. Markov processes
    12. Branching processes

11. MISCELLANEOUS AND SPECIAL TOPICS (Cream)
    0. General statistical methodology
    1. Statistical tables and charts
    2. Probability graph papers
    3. Nomograms and graphic methods
    4. Machine methods; hand and punched cards
    5. Machine methods; electronic digital
    6. Machine methods; other
    7. Monte Carlo methods
    8. Index numbers
    9. History, biography and bibliography
    10. Inventory
    11. Life-testing and reliability
    12. Teaching and training methods

There is no universally acceptable abstract calculus of probability. That is to say if one adopted a set of axioms and developed the theory of probability then the inevitable result would be some controversy and many heated arguments. Some fault would be found in the development of the theory by the purists. The purists are those who are concerned with the philosophical and correct logical foundations. They are necessary and serve a useful purpose. They are not boat rockers but keep it from sinking because they plug any holes that may be present.

Most statisticians view probability from a practical viewpoint and their attitude is that it works and does a good job, so let the purists thrash out the difficulties.

In the third topic, frequency distribution, studies are concerned with characterizing and identifying the distribution of a variable or measurable characteristic. The most famous of these distribution is the bell shaped symmetric curve known as the normal distribution. It is characterized by two parameters, namely, the mean, a measure of central tendency and the standard deviation, a measure of spread or variability.

Large standard deviation (σ is the symbol generally used) or large variance (the standard deviation squared) means the data values are more variable or spread out farther than when the standard deviation is small.

Air and water pollution measurements are examples of distribution that are not normal since they are not symmetric. These distributions are terminated on the left since there are no values below zero. Due to a few very large values they have a long right tail or as the statistician would say, skewed right.

The list of distributions used in statistics is greater than the subtitles listed. This list includes only the most important ones and a complete list of distributions developed by researchers with their properties would fill several books.

The usefulness of a frequency distribution lies in the fact that from it one can calculate the probability of occurrence of different values or set of values for the variable.

Before introducing the next topic a few preliminary remarks about the difference between a population and a sample may be in order.

A population is defined (working not a technical definition) as a body of individuals or units identified in some manner. The person specifying the population also specifies the criterion or characteristic which determines who or what is or is not a unit in the population.

Thus I can specify a population as all live humans in the State of Ohio at midnight December 31, 1972. This population is not well defined.

For example, if an airplane is within the state boundaries at midnight, then are those people in the airplane units in the population or not.

Those persons in Ohio who died near midnight and are those that the time of death is not exactly known, are undetermined units. Also note that on January 31, 1973 there will be members of the specified population who are no longer alive but are still members of the population as originally determined. I think it is safe to say that over 99% of the population is well defined.

A sample is a subset of the population. The population values as well as the number of units in it is a constant, however a sample can have any size. Two samples of the same size have different values for the measurable characteristic under consideration.

Five reasons that samples are used or preferred or are even necessary instead of the whole population are given next.

It is much easier and quicker to obtain and measure a few units in the sample as opposed to the whole population. Speed is one advantage for sampling.

Economic considerations is another advantage that needs no comment.

The third reason samples are used is when the parent population has an infinite number of units. In this case it is impossible to obtain all units, hence sampling is mandatory.

In some studies measurements are impossible to obtain. This is especially true in time series where these measurements are lost in history if they were never made.

The final reason we will consider here for using samples is that the measuring process can destroy the population. Let us suppose that we are doing a water pollution study at a certain point on a stream. If we did not use sampling techniques then there would be a dry stream bed on the one side of the sampling point. In air pollution studies, if sampling was not used then there would be no air left to breath since a vacuum would remain if the entire population or all of the atmosphere was analyzed.

Very few people have the luxury, when doing research, of measuring every unit in the population. For this reason sampling distributions per se are studied quite vigorously. On the basis of a sample, decision statements or estimates are made about the entire population. Different samples have different values or a sample varies from one sample set to another. Each sample as well as any value calculated from it has its own sampling distribution. These must be known before quantitative statements can be made.

The three most important sampling distributions are the t, F, and Chi-square. They, as well as the normal, are tabulated in almost every textbook on statistics.

In the fifth subject, estimation, the sample values are used to infer information about the entire population. For example, from a sample, one can calculate the sample mean and use this to make statements about the parent population mean. The calculated sample mean ($\bar{X}$) is an estimate of the population mean (the symbol used is the Greek letter mu).

One legitimate question that can be asked is "Is this the best possible way?" Best possible way is a fuzzy concept and must be unfuzzied before the question can be answered. That procedure produces many interesting paths as evidenced by the list of subtitles.

Hypothesis testing (or significance testing), our next topic, is a technique that is too often not really understood. It can be understood by anyone since the concept is really quite simple and is analogous to a comparison procedure familiar to everyone. A test of hypothesis is the familiar method of comparing two things (an unknown and a known) for identity or nonidentity. After the comparison has been completed then one of two conclusions is made: 1) the two things compared are not identical or 2) the things compared are identical.

In the world of statistics, the unknown in the comparison procedure is the sampled population. The known used for the comparison is a hypothetical population which is also called the mathematical model or the test assumptions.

The sampled population values are used to evaluate a statistic which can be thought of as a formula for combining the information available in the sample. The observed statistic value obtained from the sample is compared with corresponding values obtained from the mathematical model.

If the observed and expected statistic values are in disagreement then the sampled and hypothetical population disagree or are not identical. If the two statistic values agree, the populations agree or are identical.

A simple example will make the ideas clear. Assume you are sampling from normal with an unknown mean and a standard deviation equal to ten. We wish to test if the unknown mean equals five. The mathematical model or known is normal, mean = 5 and standard deviation = 10. If the conclusion of the comparison procedure is agreement between the sampled population and the mathematical model, this means both normals have equal means and standard deviations. That is equivalent to saying the two means are equal to five, or the unknown mean from the sampled population equals five. If the statistical decision was the two populations disagree then the evidence says that the mean of the sampled population doesn't equal five.

In summary, a one sentence nontechnical definition for a test of hypothesis could be: "A test of hypothesis can be defined as a method for comparing an unknown sampled population with a known population (or mathematical model) and deciding if the two populations are in agreement (or are alike) or disagreement (or are not alike)."

A longer nontechnical working definition for hypothesis testing would be as follows: A test of significance can be defined as a method of analyzing data so as to discriminate between two hypotheses. The first hypothesis is called the null hypothesis H_O which means there is no difference between sampled and hypothetical population. The difference between the two populations is equal to zero or is null. The second hypothesis is called the alternative hypothesis H_A or H_1 which should be the operational statement of the experimenters research hypothesis. Next the best test for the alternative hypothesis is selected, that is, the one that has the highest probability of saying the research hypothesis is true when it is in fact true. The probability of deciding the null hypothesis is false when it is actually true is preselected by the researcher. This value is called the alpha-error. The alpha-error specifies those values for the statistic for which the decision is agreement (or do not reject H_o or there is no difference between sampled and hypothetical populations) or there is disagreement (accept alternative or research hypothesis). The sample is now calculated and the statistic value is compared with the decisions values determined by the alpha-error. The decision is made to either: 1) do not reject the null hypothesis or, 2) reject the null hypothesis (or equivalently accept research hypothesis).

The decision to "do not reject H_o" could be correct or in error and the same remark holds for the other decision. The following Table summaries the statistical decision and the real world situation and their interrelation.

TABLE I

		Statistical Division	
		Do Not Reject H_o or Reject H_A or Populations Agree	Reject H_o or Accept H_A or Populations Disagree
REAL WORLD SITUATION	H_o is True or H_A is False	NO ERROR	ERROR (Called 1. Alpha-error 2. Type I Error 3. Error of the first kind)
	H_o is False or H_A is True	ERROR (Called 1. Beta-error 2. Type II Error 3. Error of the second kind)	NO ERROR

We continue with the simple example mentioned earlier.

$$H_0: \mu = 5 \quad \text{or} \quad H_0: \mu - 5 = 0$$

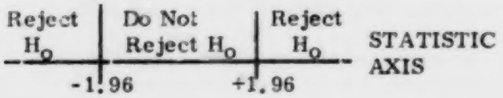
The null hypothesis states there is no difference (or equally) between the sampled mean indicated by Greek mu on the left and the hypothetical population mean of 5.

$$H_A: \mu \neq 5$$

The alternative hypothesis state the sampled mean does not equal five. The research hypothesis could be the mean is greater than five or the mean is less than five.

From a preselected alpha-error (0.05) the "do not reject H_0" region is any value for the statistic between -1.96 and +1.96 otherwise H_0 will be rejected.

Graphically we have the following situation:



$$\text{The statistic} = \frac{\sqrt{\text{sample size}\ (\bar{X} - 5)}}{10}$$

From a random sample of size 25 the sample mean was determined as $\bar{X} = 10$. The statistic is evaluated as $\sqrt{25}\,(10-5)/10 = 2.5$.

The decision is reject H_0 since 2.5 is greater than 1.96 or is in the right rejection region. There is enough evidence to say the sample population mean is not five. The two populations are in disagreement and since both are normal and have equal standard deviations the disagreement is in the mean.

Our findings could have also been interpreted as the sample mean of 10 with a sample size of 25 is very unlikely if the sampled mean was really 5 and the standard deviation was 10. The sample mean of 10 could have occured but this is a rare event or can happen with small probability.

For those readers who want an in depth nontechnical explanation of a tests of significance, the references written by this author are suggested as a beginning.

Our next topic relationship needs no introduction since every scientist understands that some sort of predictive technique is implied.

Variance analysis is next on our list. In variance analysis, the total variation displayed by a set of observations may, in certain circumstances be separated into components associated with defined sources of variation. The defined sources are used as a criterion of classification for the observations. That portion of variance which is left undefined or unexplained is called experimental error. Many standard situations can be reduced to the variance analysis form.

As an illustration, let us assume that the only contributors to the total variance in a certain water pollution parameter measurement are people, method and instruments. Let us also for simplicity assume no interaction present.

Then the total variance in the mathematical model is given by

$$\sigma_T^2 = \sigma_P^2 + \sigma_M^2 + \sigma_I^2 + \sigma_U^2$$

where the subscripts are defined as follows:

T = total variance
P = variance component due to people
M = variance component due to method
I = variance component due to instrument
U = unexplained variance

The last component has many aliases. It is also called the random variance, experimental error, unknown variance and indeterminant variance source. Indeterminant is relative since with enough time, money and experiments all variance can be identified as to its source.

With a properly designed data collection scheme the variance analysis technique would partition the sample total variance into estimates of the four components. In this way an estimate of each factor's contribution to the total variance is available. The greatest reduction in the total variance is possible by reducing that factor's contribution which is greatest. The direction to take is now clearly shown, since the numbers were made to tell on themselves.

Our ninth topic has many aliases, two examples are sampling plan and survey design. The purpose is to measure nature as it exists by taking a sample from a population. Statistical data are collected to provide a rational basis for action. The action may call for the enumerative interpretation of the data or it may call for an analytic interpretaion. These actions determine the difference between enumerative and analytic surveys.

Enumerative surveys would be classified in the upper left hand quadrant of Figure 1, while analytic surveys should appear in the lower left quadrant of that same figure.

A partial list of some considerations when samples are collected should include the following:

1. Why (objectives)
2. Who
3. What (variable)
4. Where (place)
5. When (time)
6. How
   - a Grab Sample
   - b Synoptic
   - c Composite
     - I Continuous
     - II Series of grabs composited
     - III Equal portion at equal time
     - IV Varying portion at equal time
     - V Varying portion at varying time
     - VI Equal portion at varying time
7. Size (volume)
8. Quanity (how many)
9. Cleanliness of sample container
10. Sample temperature control
11. Time lag between collection and analysis
12. Method of sample preservation
13. Which analytic method, if several are available.

In a design of experiment, our next topic, the researcher is interested in the change produced in a response by the influencing factors. For example, a researcher may be interested in the yield in bushels of wheat (the response) when different fertilizers are used in different amounts (the influencing factors under his control). The design of experiment consists of the set of rules which match treatments (or list of influencing factors to be studied) with the units (plots of ground) to which the treatments are to be applied. The principles of randomization, replication and stratification are employed and produce the many designs available.

In the eleventh topics, the adjective stochastic implies the presence of a random variable. Hence a stochastic process is one where in the system incorporates an element of randomness as opposed to a deterministic system. A time series is a set of ordered observations on a measurable characteristic taken at different points in time.

The final classification is the miscellaneous basket of topics which are not large enough for their own major classification.

VI THIRD VIEWPOINT

The final viewpoint will be the question and answer approach. This will lead to a more detailed problem classification viewpoint and tie in some of the topics discussed in the previous viewpoints.

Suppose you are interested in the B.O.D. measurements at a particular point in the Bay of San Francisco. The totality of all such measurements will be called a parent population and will be represented by some distribution as shown in Figure 3.

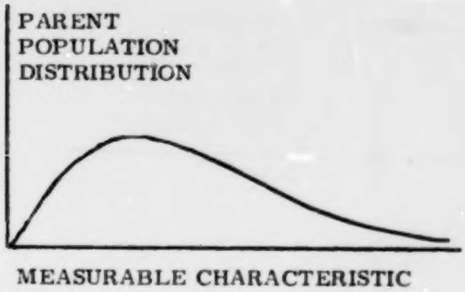


Figure 3

Three questions the researcher can ask at this point are:

1) What is the research hypothesis? Does my boss agree?

2) What is the definition of our population?

3) Am I going to study my population as is or subdivide it and study each subdivision?

Let us assume that the answer to the first question in our imaginary study has been finalized. Question one is still the best starting point in any investigation.

In this imaginary study, the answer to the second question is clearly answered but in other studies, the question is not always easily answered.

Some answers to the third question could be as follows: I will subdivide the population and compare daytime versus nighttime. An alternative choice could be, I will subdivide into the four seasons and compare.

After these three questions have been satisfactorily answered, the next question could be:

4) Will I be able to measure the entire population or take a sample?

If the answer to this question is: I will measure all the population, then the data analysis will be descriptive statistics. Statistical analysis is subdivided into two main areas, namely: descriptive, and inferential statistics.

Descriptive statistics are concerned with the problem of how you condense a large mass of data in such a way so that you summarize the available information into several succinct numbers which are simple to interpret and easier for the mind to grasp and comprehend. This type of statistic is the one students first meet in a course in statistics (See Figure 4).

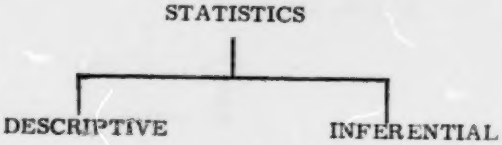


FIGURE 4

If the answer to question 4 is "take a sample" (few investigators have the luxury of measuring the entire population) then several other good questions occur like the following:

5) How big a sample do I need? Is my budget adequate for it?

6) If sample size is not fixed in advance, how will data collection be terminated?

7) How will our samples be obtained? Time, place, order, etc.

8) If our samples are not random, how will we generalize?

Statistical theory assumes random samples, hence violation of this assumption invalidates the application of the theory.

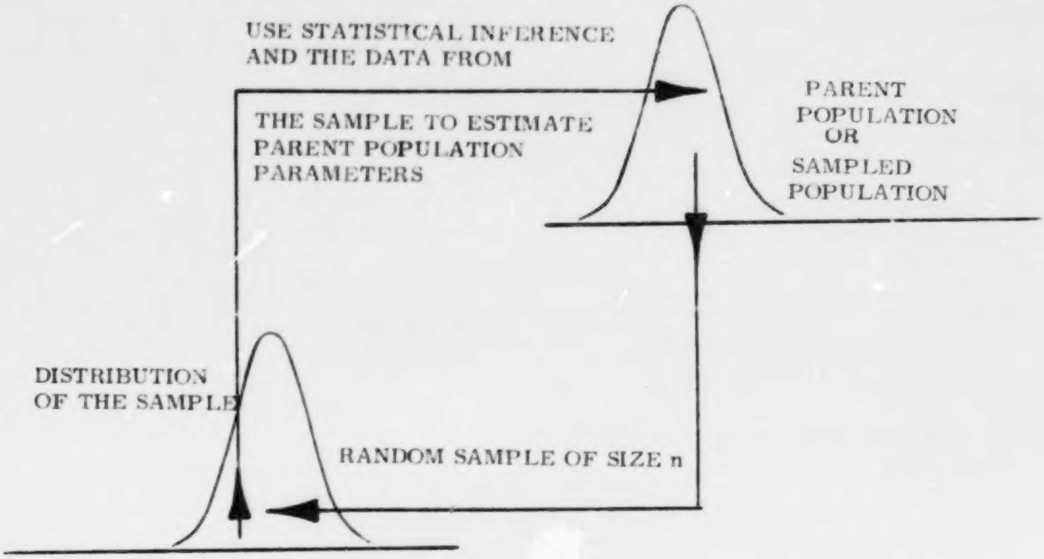

USE STATISTICAL INFERENCE
AND THE DATA FROM
THE SAMPLE TO ESTIMATE
PARENT POPULATION
PARAMETERS
PARENT
POPULATION
OR
SAMPLED
POPULATION
DISTRIBUTION
OF THE SAMPLE
RANDOM SAMPLE OF SIZE n

FIGURE 5

9) Am I going to use the sample for statistical inference with respect to estimation or hypothesis testing? (See Figures 5 and 6)

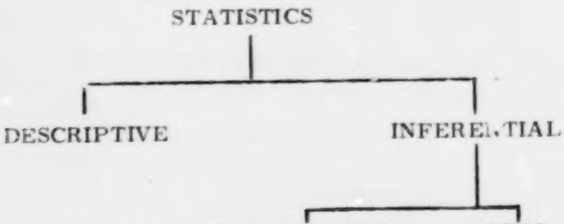

STATISTICS
DESCRIPTIVE
INFERENTIAL
HYPOTHESIS TESTING
ESTIM-
ATION

FIGURE 6

At this time a mini-example can illustrate some of the terms used. Suppose we have a population of only three values (1, 2, 3) and they all occur with equal probability.

Using descriptive statistics we calculate the population mean,

μ (population mean) $= (1+2+3)/3 = 2$

Suppose we take a random sample of size two without replacement (same value can't occur twice in our sample) and our sample values are 1, 2.

Using inferential statistics we calculate $\bar{X}$ the sample mean and use this as an estimate of the population mean.

$\bar{X}$ (estimate of μ) $= (1+2)/2 = 1.5$

The difference between the estimated value of 1.5 and the true value of 2 is due to the fact that we have a sample and is called the sampling error. Sampling errors can generally be reduced by designing better sampling schemes and taking bigger samples.

We can use the same sample to illustrate non-sampling errors. Suppose the original numbers were incorrectly read as 1 and 3 instead of 1 and 2 the correct sample values. Our estimation procedure results would be

$\bar{X}$ (estimate of μ) $= (1+3)/2 = 2$

In this case we got the right answer for the wrong reasons since the two errors compensated each other. The sampling error was -0.5 and the non-sampling (or human) error was 0.5. Non-sampling or human errors can be reduced by deligent training and careful attention to details.

It is convenient at this point to use the mini-example to explain two other terms used in statistics: biased and unbiased estimators.

Let $\bar{X}_1 = (1+2)/2 = 1.5$. The suscript means one possible $\bar{X}$. In this problem there are only three samples of size two when sampling from a parent population of 3 units when the sampling is done without replacement. The other two estimates of μ are:

$$\bar{X}_2 = (1+3)/2 = 2$$

$$\bar{X}_3 = (2+3)/2 = 2.5$$

The average of averages

$$\bar{\bar{X}} = (\bar{X}_1 + \bar{X}_2 + \bar{X}_3)/3$$

$$= (1.5 + 2 + 2.5)/3 = 2$$

is equal to the parameter $\mu = 2$.

An estimator is unbiased if its average is equal to the true value of the parameter as in this case.

A biased estimator is defined by negation, that is, if the average of the estimator is not equal to the true value then it is a biased estimator.

The difference between a statistic and a parameter are as follows: The statistic is calculated from the sample while the parameter is calculated from the parent population or the entire population. A statistic varies from sample to sample while the parameter is a constant or one number calculated for the entire population and the same units are used each time. A statistic is used to either do a test of significance or to estimate a population parameter, i.e. the sample mean is an estimate of the population mean.

Hypothesis testing can be briefly defined as a method of analyzing data so as to discriminate between two hypotheses. The one hypothesis is the research hypothesis while the other is generally the negation of the research hypothesis.

Estimation is concerned with statements about the numerical value of unknown population parameters from sample data. If the estimate is a single number, then it is called a point estimate. If an interval is calculated within which the parameter lies in a probability sense, then it is called an interval estimate. (See Figure 7)

Hypothesis testing can be subdivided into two broad types.

If the parent population is normally distributed, then the hypothesis testing is said to be parametric. If normality cannot be proven or safely assumed, then the test is said to be nonparametric. (See figure 7)

If hypothesis testing is to be applied, then some pertinent questions are:

10) What is the null hypothesis?

11) What is the alternative hypothesis?

12) What is the Type I error?

13) What is the Type II error?

14) What is the consequence of a Type I error?

15) What is the consequence of a Type II error?

At this point, other thought provoking questions can be asked.

16) What are the variables not measured that affect my observations?

17) Are we going to reject observations that seem aberrant? If so, on what basis, objective or subjective?

18) Have I given any thought to lost information?

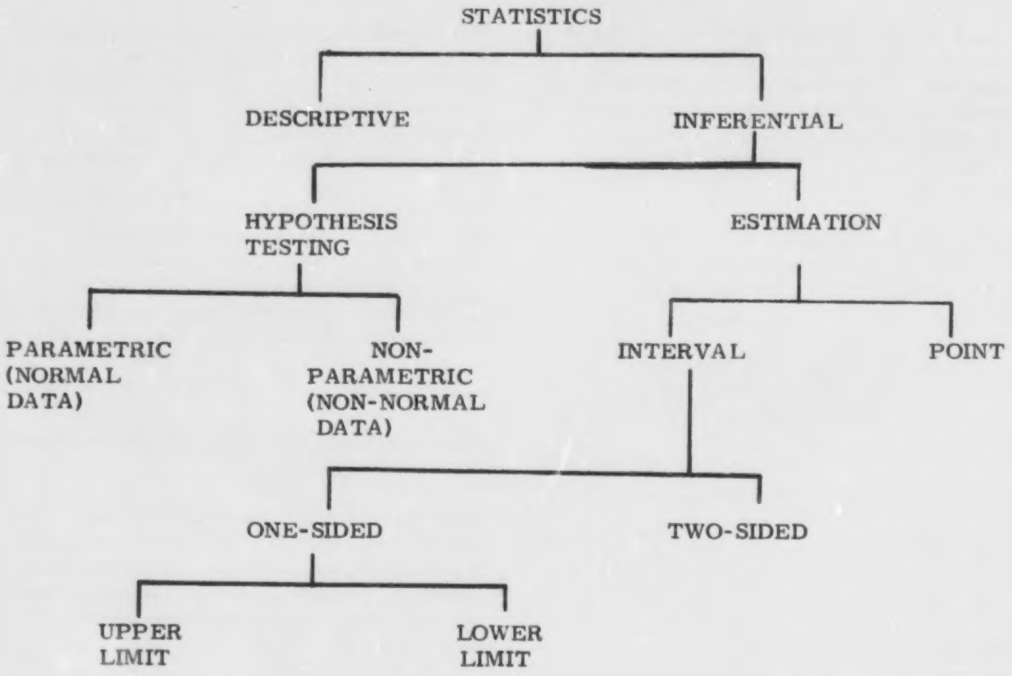

STATISTICS
DESCRIPTIVE
INFERENTIAL
HYPOTHESIS TESTING
ESTIMATION
PARAMETRIC (NORMAL DATA)
NON-PARAMETRIC (NON-NORMAL DATA)
INTERVAL
POINT
ONE-SIDED
TWO-SIDED
UPPER LIMIT
LOWER LIMIT

FIGURE 7

19) Have I given any thought to precision, accuracy and bias of measurements?

20) If there is more than one observer involved, how will I insure uniformity or separate their differences as it affects my results.

21) How will we minimize our nonsampling errors?

Some more thought-provoking questions of general nature are:

22) Will the variable observed be the one I am really interested in?

23) Is the data analysis predetermined before data collection or will this be determined after data collection?

24) What is it we are trying to do?

25) What do I have to work with?

26) What do I need to know?

27) Who else is concerned or should be?

We next consider some pointed questions related to the computer.

28) Is the computer going to be used or will the analyses be manual?

29) If a computer is going to be used, are the programs needed available?

30) If the computer programs are not available now, will they be ready when the data has been collected?

31) If the computer is going to be used and a large mass of data will have to be entered, how will the data be fed into the computer?

Some final questions for consideration are:

32) What information do we now have or is readily available?

33) What additional information do we need?

34) How will we get it?

35) How will we use it in order to assist management in making decisions?

VII RECOMMENDATIONS

There are many short courses in statistics available through EPA National Training Center. Figures 8 and 9 list the 5-day courses and the 8-hour seminars dealing with statistics.

FIGURE 8

5-DAY	
Number	Title
801	Basic Environmental Statistics
	Environmental Statistics
802	Design of Experiments
804	Nonparametric
806	Analyzing Qualitative Data
810	Applied Regression Analysis
815	Sample Size Determination
820	Survey Sampling

The five-day courses are concerned with applications while the seminars are designed to acquaint the listeners with the topics and the type problems that can be solved.

FIGURE 9

2-Day or 8-Hour Seminars	
Number	Title
899.1	Survey Sampling for Managers
899.2	Analysis of Variance and Design of Experiments
899.3	Regression and Correlation
899.4	An Introduction to Hypothesis Testing
899.5	Statistical Quality Control
899.6	Estimation and Hypothesis Testing for Normal
899.7	Nonparametric Hypothesis Testing
899.8	Introduction to and the Analysis of Categorical Data
899.9	An Introduction to Probability

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3 Santner, J. F. An Introduction to Tests of Significance Training Manual, National Training Center, DTTB, MDS, WPO, EPA.

4. Santner, J. F. Variations on a Test of Significance Training Manual, National Training Center, DTTB, MDS, WPO, EPA.

This outline was prepared by Mr. J. F. Santner, Mathematical Statistician, National Training Center, MDS, WPO, EPA, Cincinnati, OH 45268.

AERIAL RECONNAISSANCE IN POLLUTION SURVEILLANCE

I INTRODUCTION

A Definition

The word "reconnaissance" is derived from the word "reconnoiter" which means to conduct a preliminary examination or survey. Its earlier applications to engineering and military requirements has been expanded to include photomapping and interpretations of natural resources. Aerial reconnaissance can be defined as "airborne examination or survey procedures performed by heavier-than-air craft, lighter-than-air craft, or earth orbiting satellites."

B Types of Aerial Reconnaissance

1 Visual

Examination of the flight path by a human observer with no provision for permanent recording for later study. This form of aerial observation has limited use but in many cases can complement the other forms.

2 Image forming sensor

Image recording of the covered flight path where maximum advantage can be made of image interpretation techniques. Image forming sensors includes such instruments as cameras, infrared scanners, and radar.

3 Nonimage forming sensors

Nonimage forming sensors include such devices as oscilloscopes, strip charts, and dial indicators which directly indicate parameter differentials as received by the sensing elements from the target scan.

4 Combination image forming and nonimage sensors

It is advantageous at times to combine both of these techniques in order to rapidly interpret flight path imagery. This advantage is shown in Figure 1 where a heated effluent discharge is traced to a canal by infrared imagery and indicated by the "lighter" plume which parallels the shoreline. The oscilloscope phototracing indicates the temperature differential of the discharge point as compared to the offshore bay area.

C Detected images can be identified by one or more of the following criteria.

1 Size of image

2 Shape of image

3 Tonal qualities of image

4 Image profile shadowing

5 Location of image

6 Texture patterns of grouped images

7 Spatial relationship of image to surrounding bodies

D Selection of Remote Sensing Method

1 A knowledge of the electromagnetic spectrum (Figure 2) is important in order to select the most advantageous method of remote sensing. It will be noted that there are transmissibility and sensitivity limits as well as existing film limits with respect to equipment wave length patterns.

2 Remote sensing methods can be categorized into two areas.

a Source active: Utilization of an instrument which is capable of emitting a source of energy which is transmitted to the target area and emanations are received whose characteristics are dependent upon the nature of the specific target.

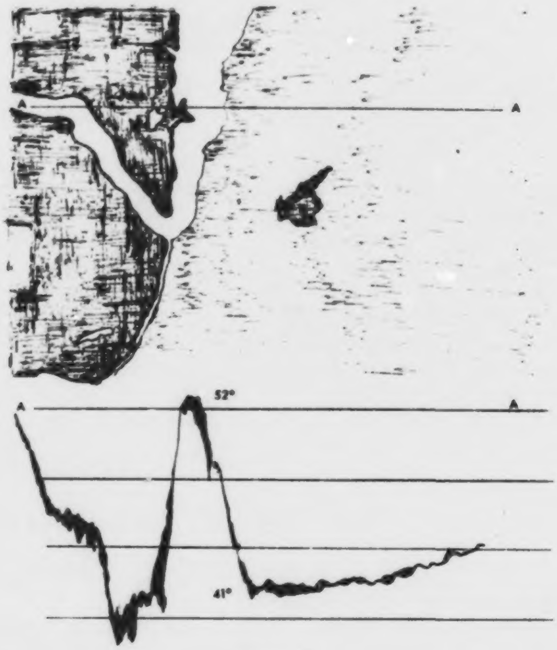

FIGURE 1

An example of such a device is the Radar set (Figure 3) or special Infrared Emitters both of which are capable of being utilized in total darkness.

b Source passive: Utilization of instrumentation which is only capable of receiving target area emanations. Such remote sensing instruments, therefore, do not transmit artificial energy sources but depend upon the sun as a source of energy which is selectively received and individually reflected by each object in the target area. Aerial photography and most of the remote sensing is by this method and the included regions of the electromagnetic spectrum are the upper portions of the UV band to the near infrared region.

3 Selection of desired wavelength is dependent upon the optimum emission capability of the instrument which has the shortest wavelength possible to sharply differentiate small objects while being long enough to preclude excessive energy "scattering" and yet not long enough so that photographic recording is impossible.

Utilization of camera systems (Multiband) exposing discrete bands of the photographic spectrum simultaneously have allowed the photo-interpreter to make discriminations which are not possible with single bands if black-and-white photography. The interpreter may use various projection devices which allow the viewing of one, two, or more images superimposed for a more sophisticated analysis capability.

II TECHNIQUES

A Radar(RAdio Detection And Ranging)

Radar is particularly useful for discerning certain types of vegetation which may not appear in the best visual quality in Panchromatic Color Film (sensitive to the

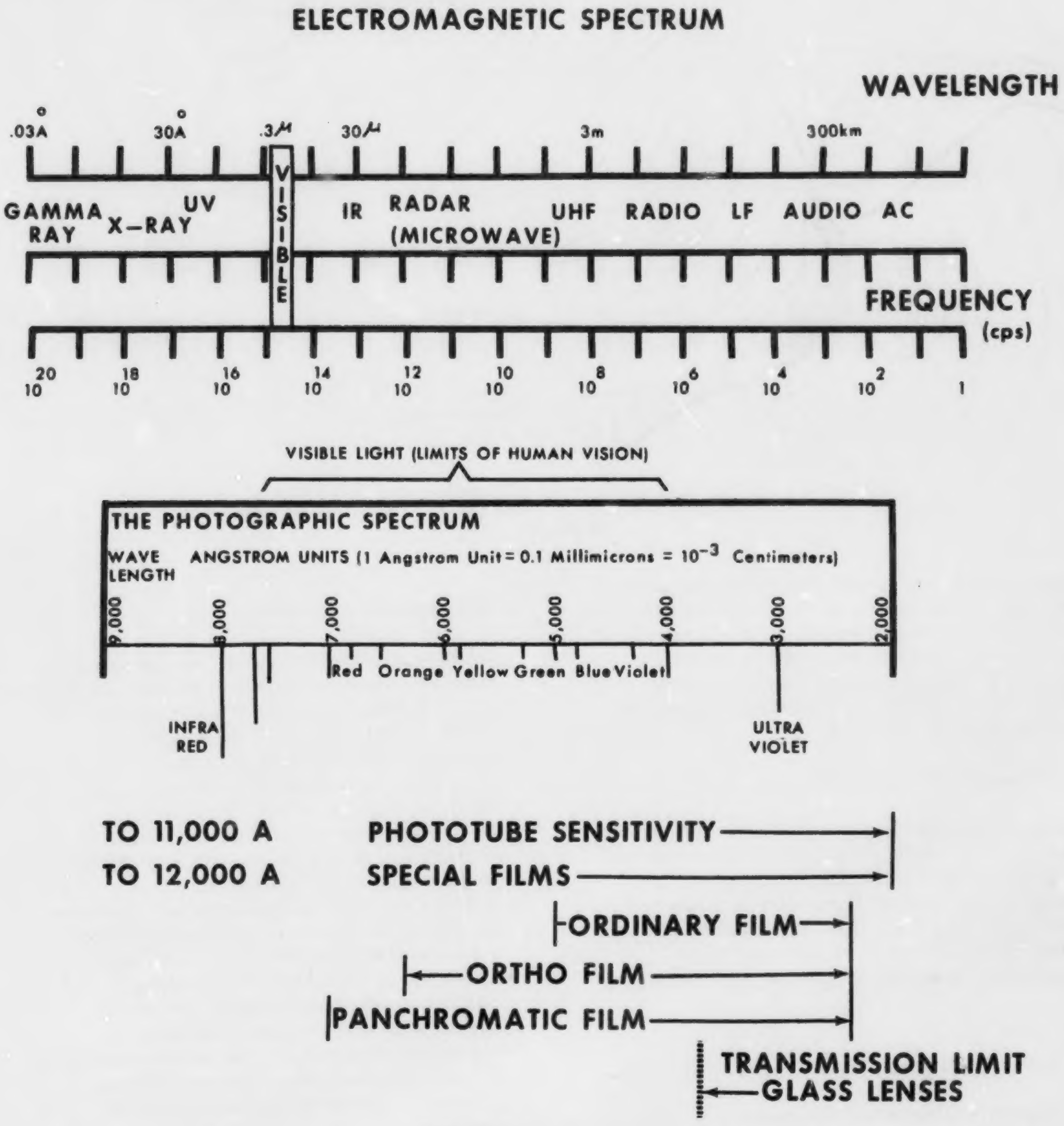
ELECTROMAGNETIC SPECTRUM
WAVELENGTH
.03Å
30Å
.3μ
30μ
3m
300km
GAMMA RAY
X-RAY
UV
VISIBLE
IR
RADAR (MICROWAVE)
UHF
RADIO
LF
AUDIO
AC
FREQUENCY (cps)
10^{20}
10^{18}
10^{16}
10^{14}
10^{12}
10^{10}
10^{8}
10^{6}
10^{4}
10^{2}
1
VISIBLE LIGHT (LIMITS OF HUMAN VISION)
THE PHOTOGRAPHIC SPECTRUM
WAVE LENGTH
ANGSTROM UNITS (1 Angstrom Unit = 0.1 Millimicrons = 10^{-3} Centimeters)
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
Red
Orange
Yellow
Green
Blue
Violet
INFRA RED
ULTRA VIOLET
TO 11,000 A
PHOTOTUBE SENSITIVITY
TO 12,000 A
SPECIAL FILMS
ORDINARY FILM
ORTHO FILM
PANCHROMATIC FILM
TRANSMISSION LIMIT
GLASS LENSES

FIGURE 2

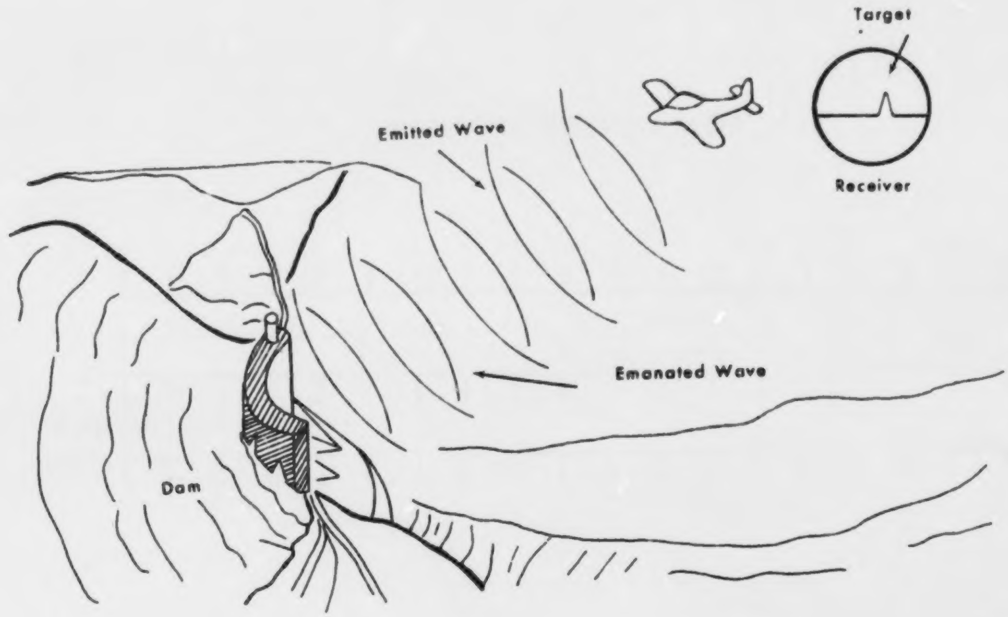

FIGURE 3

entire visible spectrum) and also has all-weather and around-the-clock capabilities. Radar in the longer wavelength ranges is capable of penetrating dense vegetative cover and this advantage has been found to be useful in detecting drainage networks and geological features.

B Conventional Photography

Conventional photography covers the entire range of the visible spectrum and employs both black-and-white film and color film. Some of the special films are capable of photographing a portion of the infrared band (the "near" infrared region) and this capability is useful in special situations.

1 Direct photomapping and panoramic scan

Figure 4 illustrated the manner in which direct photomapping differs from the panoramic scanning technique and it can be seen that continual attached mapping scans can be made of all or portions of the flight path to be analyzed.

2 Multiband photography

Multiband photography is, as its name implies, the synchronized photography of up to nine separate photographs of the same target area each of which is photographed in a different wavelength. A distinct advantage of this method is that, during photo analysis, distinct anomalies may be evident between objects appearing in the different bands and further investigation may be warranted. An example of this is shown in Figure 5 where it was observed that an anomaly in vegetative growth was apparent in the Infrared wavelength while not being apparent in the other visual spectral ranges. Further investigations proved that gravel washings in slug intervals had affected normal growth of the streamside vegetation.

3 Infrared photography

Infrared photography has been found useful in depicting certain hydrological features. Within the photographic

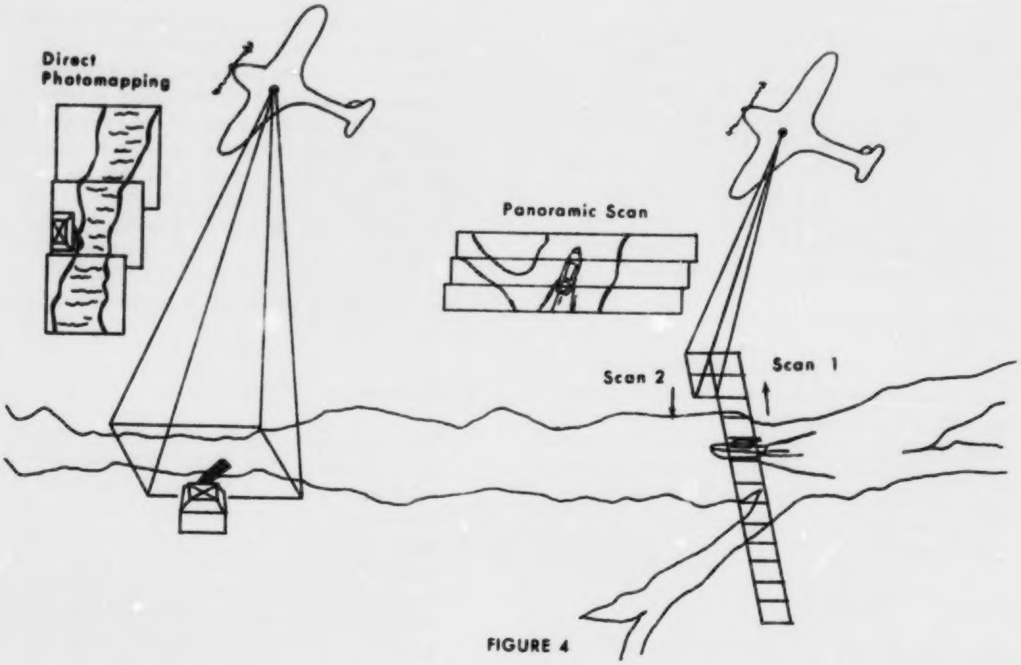
Direct Photomapping
Panoramic Scan
Scan 2
Scan 1

FIGURE 4

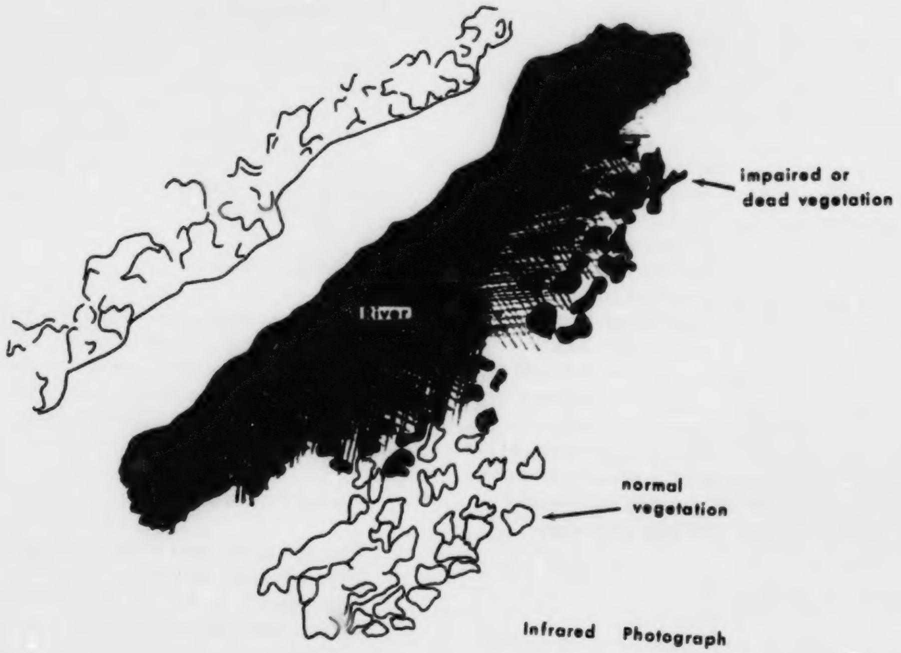
impaired or dead vegetation
River
normal vegetation
Infrared Photograph

FIGURE 5

ranges it is not a heat-measuring tool and therefore cannot detect thermal anomalies within bodies of water but instead sharply delineates shoreline and tributary features and has haze penetrating qualities. The blues appear much darker by infrared photography and the reds, greens, and yellows will show up much lighter when compared to a panchromatic color photograph.

4 Gamma Ray Spectrometer

This device functions in the very short wavelengths and has been found to have excellent detecting capabilities for radioactivity. Such a device would serve as an excellent means of searching for radioactive waste spills which have occurred either accidentally or intentionally.

C Infrared Radiometry

Infrared imagery is different than infrared photography since the tone of the imagery is directly related to the infrared radiation emitted from the target area. This type of scanner converts the normally unphotographic IR band to a thermal imagery. An optical-mechanical device is necessary since the normal thermal energy emitted by the camera itself will influence radiations received from the target area and this elimination of detector internal radiation is accomplished by miniturization and application of extremely low temperatures. One example of the usefulness of infrared imagery is shown in Figure 6 where the dark areas along the shoreline were found to be caused by cold water infiltration by a previously unknown source. Such a finding could be indicative, for example, of a new untapped freshwater source or a polluting influence from a remote source. This cold water infiltration was not evident by normal visual spectrum photography and the IR photograph would only indicate a more distinct shoreline.

D Automatic Analysis

In the analysis of large numbers of prints use can be made of photoelectric scanners wherein automatic recording of degree of brightness can be recorded on a tape printout. An example of this procedure is shown in Figure 7 where each symbol has a significance relating to "tone signature." The "D" zones are the darker areas while the other symbols relate to lighter zones. In the center the clear area is a river tributary. Future predictions are that automatic encoding can be channeled to computers wherein decisions can be made, for instance, in water management policies.

E Stereoscopic Analysis

The principles and methods developed during World War II for the stereoscopic analysis of aerial photographs has been a continuing and expanding science. Its principles are based upon the fact that the human eyes are normally spaced about 65 millimeters apart and are capable of compositing, via the optic nerve, the two separate and distinct images viewed by each orbit. Aerial stereoscopic analysis depends upon the placing of two photographs precise distances apart with precise overlapping as required by normal human visual acuity. With the aid of special stereoscopic viewing lenses the viewer is able, in most cases, to composite both of the photographs in the same manner as with normal vision and thus a sense of depth can be imparted materially enhancing the analyzing of the terrain.

III APPLICATIONS

Aerial reconnaissance in pollution surveillance can be accomplished in a variety of ways and the method chosen usually is dictated by costs involved and what is desired with regard to immediate or potential information.

A Visual Observations

The simplest and least expensive method is to visually observe the study area and this can be useful in determining sampling sites, locating previously undetermined tributaries, pinpointing visual gross

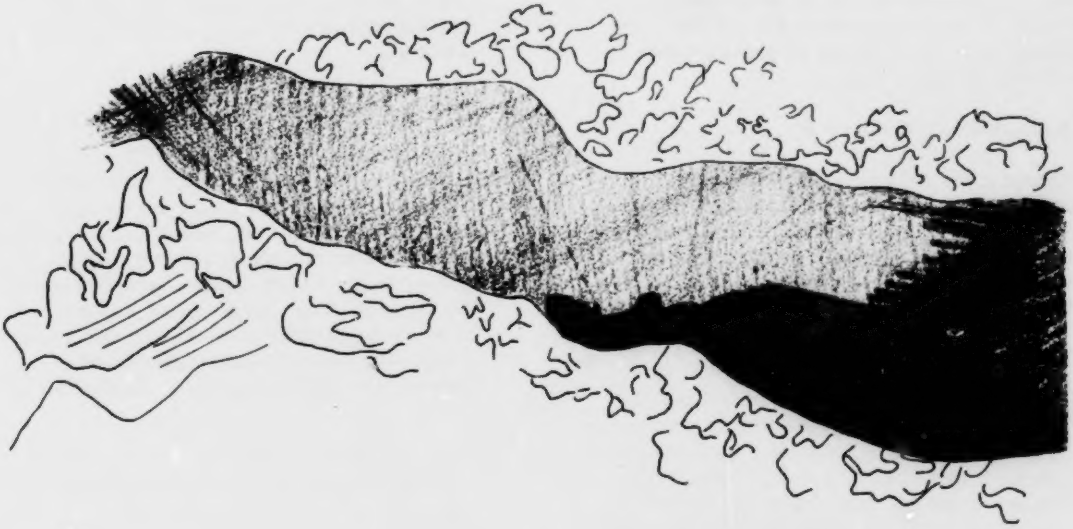

FIGURE 6

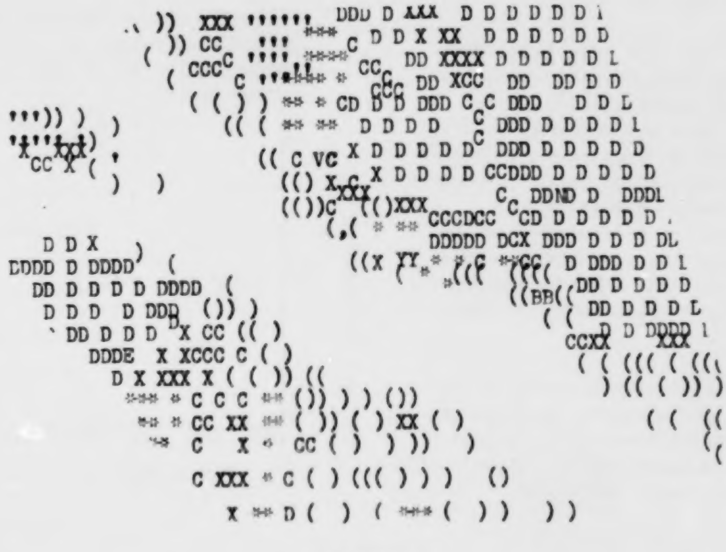

FIGURE 7

pollutant effluents, etc. To augment these observations it is usually better to have a permanent recording of the survey area in the form of photographic or imagery recordings.

B Photographic Techniques

Photographic evidence, either color, black-and-white, or special film, can be recorded in various attitudes or positions of the aircraft in order to make best use of interpretation techniques such as stereoscopic analysis.

1 Current patterns and flow velocity can be ascertained by a methcd developed by the U.S. Coast and Geodetic Survey whereby powdered aluminum is surface distributed to a wide area and subsequent photographic patterns can be analyzed. Flowing bodies can either appear as "depressions" or "elevations" depending upon direction of flow with relation to the photographing aircraft and from these "parallax" anomalies the velocity can be determined.

2 Photographic IR has found applications in the areas of detecting sharp delineations of shorelines and tributaries especially when haze is prevalent.

3 The work of Strandberg[1] concludes that "In aerial photographs, oxygen-deficient water frequently appears black, or at least darker in tone and this appearance may be caused by the incomplete reduction of wastes by anaerobic bacteria." Thus his black-and-white photographs of waterbodies with low values of dissolved oxygen (DO) following a source of waste discharge are characterized by a plume discharge followed downstream by dark water tones.

Further values ascribed to photography in this manual include color photographs dealing with fish kills where the extent of damage can be better ascessed and the use of "false" color infrared photography where algal masses can be differentiated and, again, the low DO concentration is manifested by a color anomaly.

C Imagery Techniques

1 Utilization of the "mid" and "far" infrared wavelengths in the form of "imagery" has been found useful in analyzing for thermal pollution and its concomitant circulation and diffusion patterns for which "thermographs" can be described for large water bodies.[2] It is also possible to establish naturally occurring patterns of seasonal and diurnal thermal ranges for surface waters.

2 It has been ascertained, in principle, that it is possible to identify pollutants by study of spectral emissivity characteristics for particular wavelengths and this can be of value, for instance, when one is searching for a particular pollutant and the emissivity characteristics for the waterbody can be compared with known patterns for the specific pollutant.[2]

A hypothetical situation is plotted in Figure 8 where spectrometric values of polluted water discharge are compared to a "normal" pattern of the water body.

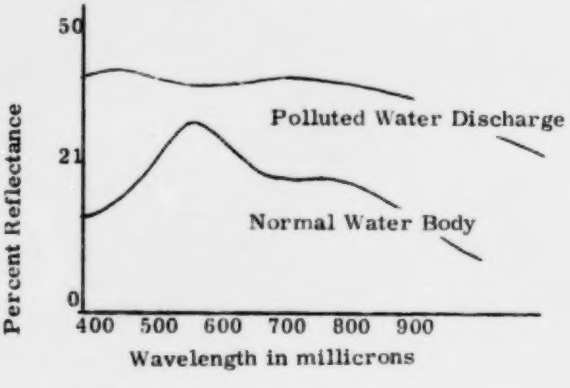

Figure 8

3 Utilization of radar and orbiting satellite platforms can produce imagery of great usefulness. The radar technique can precisely delineate shoreline characteristics without vegetative interferences and thus thoroughly image drainage basins. Satellite imagery and photography can obtain the same information but on a much broader scale and with great rapidity. Recent lines of thought have postulated that this orbiting scanner can be fitted with computing devices to render decisions upon analysis of its constant data collection.[3] Such decisions can be of immeasurable aid to the water conservationist who may require long range predictions based upon analysis of whole river basins.

IV SUMMARY

Development of aerial reconnaissance in pollution surveillance has proceeded in the areas of technique and interpretation. With regard to the techniques available most of the earlier problems have been overcome to a degree where available material is amenable to analysis. Such early problems as pitch and yaw of the aircraft, cloud cover, imagery instrumentation, etc., has been overcome to a large degree by available engineering skills and the need for improvement due to military needs. In the area of interpretation, however, much has yet to be accomplished to develop this tool as an extension of laboratory analysis which it will augment rather than replace. It is highly possible that remote sensing will take the final evolutionary form of fully automatic computerized operations. A variety of remote sensing devices can be envisioned to collectively contribute to a final printout of management decisions.

REFERENCES

1 Strandberg, C.H. "Aerioal Discovery Manual." John Wiley & Sons, Inc., New York.

2 Van Lopik, J.R., Rambie, G.S. and Pressman, A.E. "Pollution Surveillance by Noncontact Infrared Techniques." Jour. Water Poll. Control Fed., 40, 3, 425, March 1968.

3 Colwell, R.N. "Remote Sensing of Natural Resources." Scientific American. January 1968.

This outline was prepared by R. Russomanno, Microbiologist, National Training Center, WPO, EPA, Cincinnati, OH 45268.

Descriptors: Aerial Photography, Cameras, Mapping, Photogrammetry, Pollutants, Radar, Remote Sensing, Water Pollution

CASE PREPARATION AND COURTROOM PROCEDURE

I TYPES OF PROCEEDINGS IN WHICH WATER QUALITY EVIDENCE MAY BE USED

A Administrative Proceedings

1 Rule making

a Setting up of regulations having general application, e.g., stream classifications and implementation plan target dates

b Factors of safety and absolute prohibitions may be appropriate

2 Adjudications

a Determinations by agency having expertise with respect to particular discharge or discharger, e.g., approval of plans and specs and time schedule of a particular discharger

B Court Actions

1 Civil in behalf of state or federal government

a Actions to compel action or suspension of action - nuisance, health hazard, etc.,--including court action following federal conference --hearing procedure

b Violations of Water Quality Standards

c Violations of Effluent Standards or discharge permits

d Tort or contract actions relating to design and/or operation of treatment facilities

2 Criminal (dependent on content of applicable statutes).

a Discharge of specific materials

b Discharges from specific industries

c Littering

d Discharges harmful to fish and/or crustaceans

e Discharges harmful to specific types of receiving waters

f Discharges of poisons

NOTE--In some of these situations doing the act may constitute the violation; in others proof of intent or knowledge of effects may also have to be proved.

3 Private actions for damages or to compel action

a Alleged harm to plaintiff, e.g., pollution of stream killing animals

C Procedural Matters

1 See Attached sheet "Administrative and Court Proceedings" on Burden of proof, fact finding, and methods of presentation of evidence.

D Classes of Evidence - General Rules

1 Facts - direct

a The material was floating from the outfall.

2 Derived values - expert testimony - test results and/or opinion as to effects

a The D.O. was zero; the waterway was polluted; the plant can be built in 6 months.

3 Hearsay

a Joe told me

4 Relevancy

5 Admissibility vs. weight

a Even if admissible, the weight to be given is up to fact finder--credibility.

E Admissibility of Results of Sampling and Testing (Numbers)

1 Sampling

a Chain of custody

b Tags, etc.

c Containers

d Place and time

e Retention of samples (Proving that the sample represents what is at issue in the action (relevancy), that there has been no opportunity for tampering; and availability of portions for analysis by other side (non-transitory criteria)).

2 Analysis

a Who performed (Can identity of each participant be shown?)

b Admission through supervisor - custodian

c Scientific acceptance of method. Is there a particular method required to be used by the agency?

d Propriety of conduct

e Retention of bench cards and other indicia of results. (Your attorney can make arrangements to substitute copies for originals).

3 Tests

a Comparison with actual conditions

b Mathematical models - how can a computer be cross-examined?

F Admissibility of Expert Opinion on Causes and Effects

1 Who has special knowledge - and of what particular areas?

2 Indicators

3 Significance of numerical determinations or observations

4 Consistency with own prior publications and testimony

5 Have underlying facts been or need to be proved--first hand information of this and/or comparable situations.

6 Use of treatises

G Conduct on the Witness Stand

1 General

a On direct - know what counsel will ask and let him know generally what you will answer, but don't make it sound rehearsed.

b Use layman's language to extent possible.

c Listen to question and answer it to best of your ability.

d Speak so that court reporter, judge, jury, and counsel can hear you.

e Speak in language that will be understood; don't talk down.

f Answer only what you are asked --don't volunteer; however, answer with precision.

g There is nothing wrong with asking to have a question repeated or rephrased.

h There is nothing wrong with saying that you consulted with your attorney before you testified, but beware of the question "Did Mr. X tell you what to say?"

i There is nothing wrong with thinking out your answer before responding.

j You are not expected to know all the answers--if you do not know, admit it.

k Don't attempt to answer questions outside your area of personal knowledge (hearsay) or beyond your expertise. (Your may be an expert on conducting laboratory tests, but not on epidimeological inferences from results).

l Don't try to answer before the judge rules on objection.

m Show that you are an impartial dispenser of information and/or opinion, not a protagonist.

n Don't be afraid to admit what may appear to be damaging.

2 If you are testifying as an expert:

a Establish qualifications -- give information relevant to your area of expertise -- educational (including this course?), work, publications, number of times you have testified previously.

b Differentiate between physical facts (measurements and observations) and opinion (derived values).

c Be prepared to discuss theory (including assumptions) instruments used, techniques(including choice of a particular technique), physical limitations and errors, interferences.

d If experiments were conducted, be able to justify both as to theory and relevancy to this litigation.

e If you're being paid to testify, admit it.

3 Scientific personnel as advisers to counsel:

a Review and refamiliarize self with materials before you discuss with your attorney.

b Be in a position to present all facts known to you simply and concisely: Who, What, When, Where, and Why, How.

c Don't overlook facts and/or test results because you don't think they're important. Let attorney decide what he needs.

d Use of standard report forms

e Ability to recommend additional witnesses with needed specialized knowledge

f Ability to aid in cross-examination of other side's experts and reconcile opinions and/or results

g Be candid - sometimes better not to start a lawsuit or accept a settlement than lose in the end.

H Non-Verbal Presentation of Evidence

1 Exhibits - including photographs

2 Summaries

3 Business and/or government records

a Prepared contemporaneously and in usual course of activities

4 Pre-prepared direct examination

a Usually limited to actions before ICC, FPC, and other federal agencies.

I Criminal Procedure

1 Privilege Against Self Incrimination (available only to persons)

a Warning and suspects

b Effect of duty to report spills

c Effect of duty to obtain license or permit and/or furnish operating reports

d Immunity from prosecution

2 Double Jeopardy

3 Unreasonable search and seizure

a Available to persons and corporations

4 Procedures and need for arrest and search warrants --possible cause

This outline was prepared by David I. Shedroff, Enforcement Analyst, Office of Enforcement and General Counsel, Cincinnati Field Investigations Center, 5555 Ridge Avenue, Cincinnati, OH 45268.

Descriptors: Courtroom Procedure, Law Enforcement, Legal Aspects, Sampling, Water Analysis, Water Pollution, Control, Water Quality Standards

Administrative & Court Proceedings, and Excerpts from Revised Draft of Proposed Rules of Evidence for the United States Courts can be found on the following pages.

ADMINISTRATIVE & COURT PROCEEDINGS

Court or Agency	Fact Finder	Burden of Proof	Comments
State Pollution Control Agency	Agency	As per statute - usually weight of evidence.	Hearing may be conducted by hearing examiner, agency member, or full agency. Appeal may be on facts and law or law alone, depending on statute.
Rule making-adjudication			
Federal Water Pollution Control Act			
Conference	Head of agency		Reports acceptable.
Hearing	Hearing Board		Specific testimony.
Court	Judge		Uses prior material, and may take additional testimony.
Court			
Civil Case -- - for money only	Judge or jury	Weight of evidence	
- injunction			
preliminary or temporary	Judge	Must show immediate harm or danger.	Must also show likelihood of success at final hearing - bond required for non-government plaintiff.
permanent	Judge	Usually clear and convincing.	"Balance Equities"
- administrative appeal	Judge - whether "arbitrary and capricious" or substantial evidence.		Sometimes have complete new trial.
Criminal case includes penalties	Jury unless waived.	Beyond reasonable doubt.	Proof of intent may be required.

Excerpts from Revised Draft of Proposed
RULES OF EVIDENCE FOR THE UNITED STATES COURTS

GENERAL PROCEDURES

Rule 102.
PURPOSE AND CONSTRUCTION

These rules shall be construed to secure fairness in administration, elimination of unjustifiable expense and delay, and promotion of growth and development of the law of evidence to the end that the truth may be ascertained and proceedings justly determined.

Rule 101.
PRELIMINARY QUESTIONS

(a) Questions of Admissibility Generally. Preliminary questions concerning the qualification of a person to be a witness, the existence of a privilege, or the admissibility of evidence shall be determined by the judge, subject to the provisions of subdivision (b). In making his determination he is not bound by the rules of evidence except those with respect to privileges.

(b) Relevancy Conditioned on Fact. When the relevancy of evidence depends upon the fulfillment of a condition of fact, the judge shall admit it upon, or subject to, the introduction of evidence sufficient to support a finding of the fulfillment of the condition.

Rule 615.
EXCLUSION OF WITNESSES

At the request of a party the judge shall order witnesses excluded so that they cannot hear the testimony of other witnesses, and he may make the order of his own motion. This rule does not authorize exclusion of (1) a party who is a natural person, or (2) an officer or employee of a party which is not a natural person designated as its representative by its attorney, or (3) a person whose presence is shown by a party to be essential to the presentation of his cause.

Rule 611.
MODE AND ORDER OF INTERROGATION AND PRESENTATION

(a) Control by Judge. The judge may exercise reasonable control over the mode and order of interrogating witnesses and presenting evidence so as to (1) make the interrogation and presentation effective for the ascertainment of the truth, (2) avoid needless consumption of time, and (3) protect witnesses from harassment or undue embarrassment.

(b) Scope of Cross-Examination. A witness may be cross-examined on any matter relevant to any issue in the case, including credibility. In the interests of justice, the judge may limit cross-examination with respect to matters not testified to on direct examination.

Rule 613.

PRIOR STATEMENTS OF WITNESSES

(a) Examining Witness Concerning Prior Statement. In examining a witness concerning a prior statement made by him, whether written or not, the statement need not be shown or its contents disclosed to him at that time, but on request the same shall be shown or disclosed to opposing counsel.

JUDICIAL NOTICE

Rule 201.

JUDICIAL NOTICE OF ADJUDICATIVE FACTS

(b) Kinds of Facts. A judicially noticed fact must be one not subject to reasonable dispute in that it is either (1) generally known within the territorial jurisdiction of the trial court or (2) capable of accurate and ready determination by resort to sources whose accuracy cannot reasonably be questioned.

(g) Instructing Jury. The judge shall instruct the jury to accept as established any facts judicially noticed.

RELEVANCE

Rule 401.

DEFINITION OF "RELEVANT EVIDENCE"

"Relevant evidence" means evidence having any tendency to make the existence of any fact that is of consequence to the determination of the action more probable or less probable than it would be without the evidence.

Rule 402.

RELEVANT EVIDENCE GENERALLY ADMISSIBLE; IRRELEVANT EVIDENCE INADMISSIBLE

All relevant evidence is admissible, except as otherwise provided by these rules, by other rules adopted by the Supreme Court, by Act of Congress, or by the Constitution of the United States. Evidence which is not relevant is not admissible.

COMPETENCY OF WITNESSES

Rule 601.

GENERAL RULE OF COMPETENCY

Every person is competent to be a witness except as otherwise provided in these rules.

Rule 602.

LACK OF PERSONAL KNOWLEDGE

A witness may not testify to a matter unless evidence is introduced sufficient to support a finding that he has personal knowledge of the matter. Evidence to prove personal knowledge may, but need not, consist of the testimony of the witness himself. This rule is subject to the provisions of Rule 703, relating to opinion testimony by expert witnesses.

EXPERT TESTIMONY

Rule 702.

TESTIMONY BY EXPERTS

If scientific, technical, or other specialized knowledge will assist the trier of fact to understand the evidence or to determine a fact in issue, a witness qualified as an expert by knowledge, skill, experience, training, or education, may testify thereto in the form of an opinion or otherwise.

Rule 703.

BASES OF OPINION TESTIMONY BY EXPERTS

The facts or data in the particular case upon which an expert bases an opinion or inference may be those perceived by or made known to him at or before the hearing. If of a type reasonably relied upon by experts in the particular field in forming opinions or inferences upon the subject, the facts or data need not be admissible in evidence.

Rule 705.

DISCLOSURE OF FACTS OR DATA UNDERLYING EXPERT OPINION

The expert may testify in terms of opinion or inference and give his reasons therefore without prior disclosure of the underlying facts or data, unless the judge requires otherwise. The expert may in any event be required to disclose the underlying facts or data on cross-examination.

Rule 706.

COURT APPOINTED EXPERTS

(a) Appointment. The judge may on his own motion or on the motion of any party enter an order to show cause why expert witnesses should not be appointed, and may request the parties to submit nominations. The judge may appoint any expert witnesses agreed upon by the parties, and may appoint witnesses of his own selection. An expert witness shall not be appointed by the judge unless he consents to act. A witness so appointed shall be informed of his duties by the judge in writing, a copy of which shall be filed with the clerk, or at a conference in which the parties shall have opportunity to participate. A witness so appointed shall advise the parties of his findings, if any; his deposition may be taken by any party; and he may be called to testify by the judge or any party. He shall be subject to cross-examination by each party, including a party calling him as a witness.

HEARSAY

Rule 801.

DEFINITIONS

The following definitions apply under this Article:

(a) Statement. A "statement" is (1) an oral or written assertion or (2) nonverbal conduct of a person, if it is intended by him as an assertion.

(b) Declarant. A "declarant" is a person who makes a statement.

(c) Hearsay. "Hearsay" is a statement, other than one made by the declarant while testifying at the trial or hearing, offered in evidence to prove the truth of the matter asserted.

Rule 802.

HEARSAY RULE

Hearsay is not admissible except as provided by these rules or by other rules adopted by the Supreme Court or by Act of Congress.

Rule 803.

HEARSAY EXCEPTIONS: AVAILABILITY OF DECLARANT IMMATERIAL

The following are not excluded by the hearsay rule, even though the declarant is available as a witness:

(5) Recorded Recollection. A memorandum or record concerning a matter about which a witness once had knowledge but now has insufficient recollection to enable him to testify fully and accurately, shown to have been made when the matter was fresh in his memory and to reflect that knowledge correctly. If admitted, the memorandum or record may be read into evidence but may not itself be received as an exhibit unless offered by an adverse party.

(6) Records of Regularly Conducted Activity. A memorandum, report, record, or data compilation, in any form, of acts, events, conditions, opinions, or diagnoses, made at or near the time by, or from information transmitted by, a person with knowledge, all in the course of a regularly conducted activity, as shown by the testimony of the custodian or other qualified witness, unless the sources of information or other circumstances indicate lack of trustworthiness.

(18) Learned Treatises. To the extent called to the attention of an expert witness upon cross-examination or relied upon by him in direct examination, statements contained in published treatises, periodicals, or pamphlets on a subject of history, medicine, or other science or art, established as a reliable authority by the testimony or admission of the witness or by other expert testimony or by judicial notice. If admitted, the statements may be read into evidence but may not be received as exhibits.

IDENTIFICATION OF PERSONS AND SAMPLES

Rule 901.

REQUIREMENT OF AUTHENTICATION OR IDENTIFICATION

(a) General Provision. The requirement of authentication or identification as a condition precedent to admissibility is satisfied by evidence sufficient to support a finding that the matter in question is what its proponent claims.

(b) Illustrations. By way of illustration only, and not by way of limitation, the following are examples of authentication or identification conforming with the requirements of this rule:

(1) Testimony of Witness with Knowledge. Testimony that a matter is what it is claimed to be.

(3) Comparison by Trier or Expert Witness. Comparison by the trier of fact or by expert witnesses with specimens which have been authenticated.

(9) Process or System. Evidence describing a process or system used to produce a result and showing that the process or system produces accurate result.

ADMISSIBILITY AND PROOF OF SPECIAL MATTERS

Rule 406.

HABIT; ROUTINE PRACTICE

(a) Admissibility. Evidence of the habit of a person or of the routine practice of an organization, whether corroborated or not and regardless of the presence of eyewitnesses, is relevant to prove that the conduct of the person or organization on a particular occasion was in conformity with the habit or routine practice.

(b) Method of Proof. Habit or routine practice may be proved by testimony in the form of an opinion or by specific instances of conduct sufficient in number to warrant a finding that the habit existed or that the practice was routine.

Rule 612

WRITING USED TO REFRESH MEMORY

If a witness uses a writing to refresh his memory, either before or while testifying, an adverse party is entitled to have it produced at the hearing, to inspect it, to cross-examine the witness thereon, and to introduce in evidence those portions which relate to the testimony of the witness.

Rule 1006.

SUMMARIES

The contents of voluminous writings, recordings, or photographs which cannot conveniently be examined in court may be presented in the form of a chart, summary, or calculation. The originals, or duplicates, shall be made available for examination or copying, or both, by other parties at a reasonable time and place. The judge may order that they be produced in court.

GENERAL PROCEDURE FOR PESTICIDE ANALYSIS

The increasing application of pesticides in large-scale agriculture and in forest areas can contribute to the presence of these toxic materials in a water supply. Contamination can occur through drainage from the surrounding terrain, precipitation from the atmosphere, accidental spills of pesticides in the watershed area, or a cross connection on a distribution system.

Experimental investigations have demonstrated that conventional water treatment processes (in the absence of carbon) are capable of coping with DDT but are considerably less effective against most of the other chlorinated hydrocarbons.

I GENERAL DISCUSSION

A Principle

Gas chromatography is the most useful single technique presently available to the residue analyst. It differs from paper and thin-layer chromatography in that a permanent gas such as nitrogen, argon, helium, or hydrogen is used as the mobile phase. The stationary phase is prepared by impregnating an inert solid with a non-volatile organic liquid. A free-flowing powder is formed which is then packed into a glass or metal tube, usually about one quarter inch in diameter and six feet long. The tube is installed in a heated cabinet, and a stream of gas forced through it at a constant rate. A solution containing a mixture of pesticides is injected into the hot column at the end nearest the gas inlet. The pesticides vaporize and are carried through the column by the moving gas. They travel through the column at different rates owing to differences in their partition coefficients between the liquid stationary phase and the mobile gas phase. Consequently, they emerge from the far end of the column at different times, and pass through a detector which automatically measures their concentrations. The response of the detector is fed into a strip chart recorder so that the presence of each compound is registered as a peak and is measured as it emerges from the chromatograph. Several detectors are available which are capable of indicating the presence of pesticides, but the most sensitive of these is the electron capture detector. This detector yields extremely high responses to halogen-containing compounds and some organic phosphates, but is relatively insensitive to many compounds which do not contain a halogen.

B Interference

In addition to some organic phosphates, many oxygenated compounds, particularly those containing carbonyl groups, give high response to electron capture. Furthermore, as with other chromatographic methods, it is not always possible to be certain that the peak in question is not due to another insecticide of similar retention time which has not been previously studied. In such cases, one of the best techniques for positive identification of pesticide residues is by the use of micro-infrared techniques after trapping fractions from the gas chromatographic column. Unfortunately, this is a tedious and exacting process that requires a great deal of skill, knowledge and equipment that is not generally available. Therefore, in lieu of positive identification by infrared, for routine analysis most residue laboratories use some additional technique such as prior fractionation on a florisil or alumina column, or thin-layer chromatography. The most convenient technique, however, is to compare relative retention times on a minimum of two gas chromatographic columns. The tentative identification made on the one column (polar) is then confirmed by rechromatographing the sample on a second, nonpolar column.

C Detection Limit

Preliminary data indicate the detectable concentrations for chlorinated hydrocarbon pesticide using electron capture gas chromatography is of the order of 0.01 μg/l.

II EQUIPMENT

A Separatory Funnels - 2 liter capacity with teflon stopcock and stopper.

B Erlenmeyer Flasks - 500 ml capacity.

C Chromatographic Column - 20 × 400 mm column with coarse fritted plate on bottom and teflon stopcock.

D Conical Centrifuge Tubes - 15 ml capacity.

E Aluminum Tubing - 1/4" O.D. and 0.032" wall thickness.

F Pyrex Glass Wool.

G Gas Chromatograph - Equipped with dual columns and electron capture detector.

H Strip Chart Recorder - 1 mv.

I Syringes - 10 μl.

J Glassware - All glassware used in pesticide analysis should be rinsed successively with chromate cleaning solution, running tap water, distilled water, and finally several times with redistilled solvent (hexane, petroleum ether or ethyl acetate).

III REAGENTS

A Hexane - AR grade and redistilled (Burdick and Jackson Laboratories, Inc. or Mallinckrodt Nanogram Grade).

B Hydrochloric acid, conc.

C Sodium Sulfate - Anhydrous, granular.

D Florisil - Activated at 1200° F and stored at 130° C.

E Petroleum Ether - Redistilled, bp 30-60° C (Burdick and Jackson Labs., Inc. or Mallinckrodt Nanogram Grade).

F Diethyl Ether - redistilled.

G Mixed Ethers - Mix 1 volume diethyl ether with 3 volumes redistilled petroleum ether.

H DC-200 Silicone Fluid - 12,500 centistokes (Analabs, Inc., 9 Hobson Avenue, Hamden, Connecticut).

I Anakron ABS - 80/90 mesh (Analabs, Inc., 9 Hobson Avenue, Hamden, Connecticut).

J SE-30 silicone gum rubber (Analabs, Inc., 9 Hobson Avenue, Hamden, Connecticut).

K QF-1, fluorosilicone (Analabs, Inc., 9 Hobson Avenue, Hamden, Connecticut).

L Ethyl Acetate - Redistilled (Burdick and Jackson Laboratories, Inc. or Mallinckrodt Nanogram Grade).

M Nitrogen Gas - Purified grade.

N Molecular Sieve - 5A (Analabs, Inc. 9 Hobson Avenue, Hamden, Connecticut).

O Stock Pesticide Solution - Dissolve 100 mg of the pesticide of interest and dilute with ethyl acetate to 100 ml to form a solution containing 1.00 mg per 1.00 ml. If necessary, prepare mixtures of pesticides at this same concentration.

P Standard Pesticide Solution - Dilute 1.00 ml stock pesticide solution to 100 ml with ethyl acetate: 1.00 ml = 10.0 μg pesticide.

IV EXTRACTION OF PESTICIDES

A Measure exactly one liter of the water sample into a 2-liter separatory funnel equipped with a teflon stopcock. Acidify the sample with 0.5 ml of concentrated hydrochloric acid and extract successively with 100, 50, and 50 ml of redistilled hexane.

TABLE 1

ELECTRON CAPTURE GAS CHROMATOGRAPHY
USING NONPOLAR COLUMNS

(10% DC 200 on Anakrom ABS)

Pesticide	Relative Retention Time*	Pesticide	Relative Retention Time*
methyl ester of 2,4-D	0.29	β-chlordane	1.60
benzene hexachloride	0.38, $\underline{0.47}$	butoxy ethyl ester of 2,4-D	1.78
isopropyl ester of 2,4-D	0.41	DDT (Tech.)	1.81, 2.44, $\underline{3.03}$
lindane	0.47	DDE	1.81
isobutyl ester of 2,4-D	0.61	dieldrin	1.83
isopropyl ester of 2,4,5-T	0.66	*o*,*p*'-TDE	1.86
n-butyl ester of 2,4-D	0.71	isooctyl ester of 2,4-D	2.00, $\underline{2.37}$
ronnel	0.78	endrin	$\underline{2.05}$, 2.29
heptachlor	0.79	ethyl hexyl ester of 2,4-D	2.07
parathion	0.98	Perthane	2.11
Chlorthion	0.99	endosulfan II	2.16
aldrin	1.00	TDE	2.33
Kelthane	1.01	chlorobenzilate	2.43
n-butyl ester of 2,4,5-T	1.08	*o*,*p*'-DDT	2.44
Suphenone	1.24	isooctyl ester of 2,4,5-T	2.70, 3.10, 3.65
heptachlor epoxide	1.24	carbophenothion	2.83
chlorbenside	1.36	Dilan	2.83, $\underline{3.37}$
TDE olefin	1.42	butoxy ethyl ester of 2,4,5-T	2.85
α-chlordane	1.43	*p*,*p*'-DDT	3.03
Perthane olefin	1.47	methoxychlor	4.5
propylene glycol butylether ester of 2,4-D	1.51	Strobane	1.07-3.55
		toxaphene	1.18-4.97
endosulfan I	1.58	tetradifon	5.4
ovex	1.58	coumaphos	9.5

* Aldrin reference; retention time approximately 4.25 min. When more than one peak is present, the major peak is underlined.

B Rinse the drained sample container with three 50-ml aliquots of hexane; the first two rinse volumes to serve as the first extraction volume (100 ml) for the sample and the third rinse serving as the second extraction volume.

C Shake the sample only moderately for four minutes because vigorous shaking may cause severe emulsions, particularly in waters of high organic content and/or high turbidity.

D Combine the extracts in a 500-ml erlenmeyer flask and dry by pouring through a two-inch column of anhydrous sodium sulfate contained in a small funnel plugged with cotton.

E Rinse the column three times with approximately 5 ml of hexane and add the rinsings to the extract in a clean, dry 500-ml erlenmeyer.

F Evaporate the extract nearly to dryness over a steam bath using a current of dry air. Do not overheat the residue or leave the erlenmeyer on the steam bath after all the solvent has evaporated.

V FLORISIL COLUMN CLEAN-UP. This step may be unnecessary when working with finished waters. If so, proceed to step V-D.

A Mount a 20 × 400 mm chromatographic column and fill to a depth of 4 inches with activated florisil with topping; top with about 1 inch of anhydrous granular Na_2SO_4. Add 30 to 40 ml petroleum ether to pre-wet adsorbent and when petroleum ether has just sunk in close the teflon stopcock.

B Dissolve the hexane extract residue in 10 ml of redistilled petroleum ether and add it to the florisil column. Open the stopcock, wash the erlenmeyer with two 10-ml portions of redistilled petroleum ether and add the washings to the column. When the level of the washings is near the top of the column packing, elute the sample by passing 200 ml mixed ethers through the column.

C Collect the eluant in a 500-ml erlenmeyer and then evaporate the eluant to about 5 ml on a steam bath with a current of dry air.

D Transfer sample to a 15-ml conical centrifuge tube, using three rinsings of petroleum ether (1 ml each).

E Evaporate the solvent nearly to dryness. Rinse the walls of the tube with (0.5 ml) diethyl ether and again evaporate nearly to dryness.

F Dissolve the residue and make up to 1 ml in hexane.

VI ELECTRON CAPTURE GAS CHROMATOGRAPHY

Even with sophisticated chromatographic equipment embodying selective detectors, successful residue analysis depends primarily on the chromatographic column. The identification of the pesticide residues in a sample extract rests to a great extent on the retention times of pesticide standards. Furthermore, for quantitative analysis the pesticides contained in complex mixtures must be completely resolved from each other. Therefore, at least two different columns are required, and tentative identifications made by comparing retention times on a nonpolar column are confirmed by rechromatographing the sample on a polar column. Relative retention times of a number of pesticides on two such columns are included in Tables 1 and 2. However, each laboratory should compile tables of retention times for their own instruments and columns.

A Treatment of Nonpolar Packing

Dissolve 2 g DC-200 silicone fluid in 50 ml ethyl acetate in a 250-ml beaker. Slowly add 20 g Anakrom ABS, with stirring, to the other ingredients in the beaker; making sure that all the particles are thoroughly wetted. Evaporate the solvent under a stream of nitrogen or clean dry air while stirring the slurry continuously. When the packing is just damp, spread out in a thin layer on the bottom of a shallow tray in order to air dry. Finally, dry in an oven at 100° C.

B Treatment of Polar Packing

Dissolve 0.8 g SE-30 silicone and 1.2 g QF-1 silicone in ethyl acetate and proceed as described above.

C Preparation of Separate Nonpolar and Polar Columns

Pack 1/4" diameter aluminum column, six feet long, by inserting a glass wool plug into one end of the tube and attaching the same end with a piece of rubber tubing to a vacuum pump or line. Insert the other end of the tube into the packing material so that the material is sucked into the tube. Tap or vibrate the tube to assure a more uniform packing, continuing this operation until the column is filled. Then plug the column with glass wool at the other end.

TABLE 2

RELATIVE RETENTION TIMES OF PESTICIDE STANDARDS ON POLAR COLUMNS USING ELECTRON CAPTURE DETECTOR

(SE-30 plus QF-1)

Pesticide	Relative Retention Time
methyl ester of 2,4-D	0.44
benzene hexachloride (Tech.)	0.45, 0.57, 0.68
methyl ester of silvex	0.46
lindane	0.57
methyl ester of 2,4,5-T	0.67
heptachlor	0.81
aldrin	1.00
Kelthane	1.38
heptachlor epoxide	1.50
endosulfan I	1.72
p,p'-DDE	1.78
Perthane	2.13
dieldrin	2.31
o,p'-DDT	2.62
TDE	2.89
carbophenothion	3.04
endosulfan II	3.07
p,p'-DDT	3.54
endrin	4.13, 6.38
methoxychlor	5.44
tetradifon	8.70

Aldrin reference. When more than one peak is present, the major peak is underlined.

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D Conditioning of the Columns

Install the packed columns in the gas chromatograph oven or other suitable oven and turn on the nitrogen carrier gas. If the column is installed in the gas chromatograph oven, do not connect to the detector but rather vent to the atmosphere to prevent buildup of column bleed in the detector system. Adjust the flow of carrier gas to about 50 ml per minute, and raise the column temperature to 250° C for the nonpolar and 225° C for the polar column. Allow the columns to condition at this temperature for a minimum of sixty hours.

E Molecular Sieve

To remove the moisture and other minor impurities from the nitrogen gas, insert a 1/4" O.D. column six feet in length which has been packed with Molecular Sieve 5A, in the line between the gas cylinder and the gas chromatograph. This is particularly important because trace amounts of moisture reduce the sensitivity of the electron capture detector.

F Standard Curves

Prepare standard curves for all pesticides to be determined. Set up the pesticide series in the range of 0.1 to 1.0 ng per 10 µl by diluting 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 ml of the applicable standard pesticide solution to 100 ml with ethyl acetate. Inject these standards into the chromatograph under the same conditions used for the samples. Plot ng of pesticide against peak height to obtain a standard curve. Run check standards immediately after each sample in order to detect any changes in detector response.

G Operating Parameters

Operate the nonpolar column at a column temperature of 190° C, injection block at 230° C, and electron capture detector at 200° C. Maintain the flow rate of nitrogen through the column at 120 ml/min. Adjust the detector purge flow of 30 ml/min. in order to obtain optimum detector response; namely, a total flow through the detector of 150 ml/min. Determine optimum operating conditions for the electron capture detector in the individual laboratory by varying the voltage applied to the detector to find a region where sensitivity is good and response is linear. If necessary, condition the column by repeated injection of mixtures as well as individual pesticides in order to obtain relevant retention times and consistent peaks. A typical gas chromatogram obtained with the electron capture detector and the nonpolar column is shown in Figure 1.

H Operate the polar column at a column temperature of 180° C, injection block of 210° C and the detector at 190° C. Regulate the flow rate of nitrogen through the column at 100 ml/min. and the detector purge at 50 ml/min. Determine the optimum voltage and condition the column as was done for the nonpolar column.

I Determination of Extraction Efficiency

Add known amounts of pesticide standard solution to control samples and carry through the extraction and cleanup procedure in order to ascertain the percentage recovered. Apply the resulting correction factor to the final calculation of the quantity of pesticide in an unknown sample.

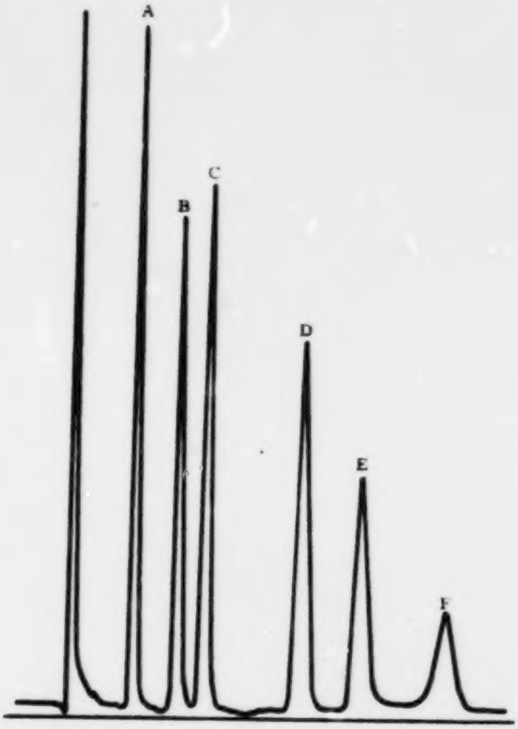

Figure 1. Electron Capture Gas Chromatogram of Pesticide Standards on Nonpolar Column

- **A. Lindane**
- **B. Heptachlor**
- **C. Aldrin**
- **D. DDE**
- **E. TDE**
- **F. p,p'-DDT**

VII CALCULATION

$$\mu g/l \text{ pesticide} = \frac{A \times B \times C}{D \times E \times F}$$

A = ng of pesticide found in solution injected in gas chromatograph.

B = volume of cleanup extract in ml.

C = dilution of extract prior to injection in gas chromatograph.

D = volume in µl injected in gas chromatograph.

E = efficiency of extraction expressed as a decimal.

F = volume of original sample in milliliters.

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This outline was prepared by Earl F. McFarren, Chief, Analytical Quality Control, Bureau of Water Hygiene, EPA, 5555 Ridge Avenue, Cincinnati, OH 45213.

Descriptors: Chemical Analysis, Gas Chromatography, Pesticide Residues, Pesticides, Water Analysis

GENERAL PROCEDURE FOR ATOMIC ABSORPTION ANALYSIS

Magnesium, copper, iron, and zinc can be determined by atomic absorption with a sensitivity limit that is lower than the established drinking water standards. Therefore, in evaluating the safety of drinking water, these metals can be determined by simply aspirating the aqueous solution into the flame. Chromium, manganese, and silver can likewise be determined, although the precision and accuracy may not be as good. The determination of cadmium and lead at the concentration set for drinking water may be difficult. However, at these lower concentrations these metals can be determined by complexing with a chelating compound, extracting the chelate into an organic solvent and aspirating the organic phase directly into the flame.

I GENERAL DISCUSSION

A Principle

Atomic absorption resembles emission flame photometry in that a sample is aspirated into a flame and atomized. The major difference rests in the fact that flame photometry measures the amount of light emitted, whereas in atomic absorption spectroscopy a light beam is directed through the flame, into a monochromator, and onto a detector which measures the amount of light absorbed. Absorption is more sensitive because it depends upon the presence of free unexcited atoms and in the usual flames the ratio of unexcited to excited atoms at a given moment is very high. Since each metallic element has its own characteristic absorption wavelength, a source lamp composed of that element is employed for each element and hence the method is relatively free of spectral or radiation interferences. The amount absorbed in the flame is proportional to the concentration of the element in the sample.

B Interference

The most troublesome type of interference is usually termed "chemical" and results from the lack of absorption from atoms bound in molecular combination in the flame. This phenomenon can occur when the flame is not sufficiently hot to dissociate the molecule (in the case of phosphate interference with magnesium) or because the dissociated atom is immediately oxidized to a compound that will not dissociate further at the temperature of the flame. The interference of phosphate in the magnesium determination can be overcome by the addition of lanthanum. In the determination of manganese, the addition of calcium similarly eliminates the silica interference.

II APPARATUS

A Atomic absorption spectrophotometer

B Autoclave

C Glassware - All glassware, including sample bottles, should be rinsed with 1 + 1 HNO_3 followed by deionized distilled water to avoid contamination errors.

III REAGENTS

A Deionized distilled water - Prepare by passing distilled water through a mixed bed of ion-exchange resins. Use deionized distilled water for the preparation of all reagents, calibration standards, and as dilution water.

B Hydrochloric acid, conc.

C Stock metal solution - Prepare as directed in Table 1.

TABLE 1

Metal	Wavelength mμ	Stock solution, page reference[a]	Sensitivity (ppm per 1%)[b]
Zinc	213.8	318	0.04
Chromium	357.9	124	0.15
Cadmium	228.8	69	0.04
Copper	324.7	131	0.20
Magnesium[c]	285.2	171	0.015
Manganese[d]	279.4	174	0.15
Silver	328.1	268	0.10
Lead	283.3	164	0.50
Iron	248.3	157	0.30

[a] SMEWW, 12 Ed.

[b] ppm of metal for 1% absorption in an aqueous solution.

[c] Add lanthanum to overcome phosphate interference.

[d] Add calcium to overcome silica interference.

D Standard Solutions - In general, prepare a series of standard solutions containing 0.01, 0.02, 0.04, 0.06, 0.08 and 0.10 mg/l by appropriate dilution of the stock solution.

E Fuel and Oxidant - Use an air-acetylene flame for all the metals in Table 1.

F Special reagent for the determination of magnesium - Stock lanthanum solution: Dissolve 58.65 g lanthanum oxide, La_2O_3, in 250 ml of concentrated HCl. Dilute to 1000 ml with distilled water to produce a solution containing 5 g La per 100 ml. Add the acid slowly until the material is dissolved. Add sufficient amounts of this solution to the magnesium standards and unknowns in order to obtain a final working concentration of 1 g La per 100 ml volume.

G Special reagent for the determination of manganese - Stock calcium solution: Dissolve 2.4752 g calcium carbonate in the minimum volume of hydrochloric acid, and dilute to one liter to obtain a solution containing 1000 mg/l calcium. Add sufficient amounts of this solution to manganese standards and unknowns to produce a final working concentration of 50 mg/l.

H Special reagents for the determination of low concentrations of lead and cadmium.

1 Methyl isobutyl ketone

Check for contamination by burning in the flame. If contaminated, wash one volume of methyl isobutyl ketone with five volumes of 1 + 99 hydrochloric acid solution. Remove the excess acid by washing with deionized distilled water.

2 Ammonium pyrrolidine dithiocarbamate solution

Dissolve 4 g in 100 ml distilled water. (K and K Laboratories, Inc., Plainview, N.Y.)

IV PROCEDURE

A Pretreatment of Sample

Acidify all water samples with hydrochloric acid (1 ml concentrated hydrochloric acid per 100 ml sample) and autoclave at 250° F for one hour to solubilize particulate matter.

B Precautions

Install the spectrophotometer on a level bench or table, with the burner under a vent.

C Instrument Operation

Because the differences between the makes and models of satisfactory atomic absorption spectrophotometers render impossible the formulation of detailed instructions applicable to every instrument, follow the manufacturer's operating instructions. As a rule, choose the correct hollow cathode lamp, install, and align in the instrument; position the monochromator at the correct wavelength (Table 1); select the proper monochromator slit width; set the light source current according to the manufacturer's recommendation; light the flame and regulate the flow of fuel and oxidant; adjust the burner for maximum percent absorption and stability; and balance the photometer. Then run the standards which are needed for the working curves and plot the concentrations of the standards against absorbance. If necessary on some instruments, convert percent absorption to absorbance. (The calibration curves are nearly linear for most elements.) Next run the samples and read the concentration of each sample from the calibration curve. For best results run standards each time a sample or series of samples are run.

D Special procedural steps for low concentrations of lead and cadmium

Acidify 200 ml water sample to a pH of 2.2 to 2.8 by adding hydrochloric acid. Add 1 ml ammonium pyrrolidine dithiocarbamate solution and 5 ml methyl isobutyl ketone, and shake for two minutes manually. If an emulsion forms at the interface of the two layers, centrifuge for 10 minutes. Repeat the extraction with a second 5 ml of methyl isobutyl ketone and combine the two extracts. Determine metal in the organic phase against standards which have similarly been extracted. If a precipitate forms in the solvent phase during the first extraction, add an additional 5 ml of methyl isobutyl ketone and repeat the extraction with a second 10 ml. This, of course, will reduce the concentration factor and sensitivity of the method and should not be done unless necessary.

E Aspiration of an organic solvent into the flame

When working with organic solvents the fuel-to-air ratio must be reduced as the burning of the organic solvent contributes to the fuel supply and in addition to lifting the flame off the burner may produce an undesirable luminescent flame. In setting the fuel-to-air ratio of the gas mixture at the burner, start with the settings recommended by the manufacturer for the analysis and gradually reduce the fuel flow (while the solvent is being aspirated) until the flame is as blue as possible and does not lift off the burner. Generally, the fuel flow can be cut almost in half.

V CALCULATION

A For aqueous sample refer directly to the calibration curve.

mg/l = (mg/l in aliquot) × D

$$D = \text{dilution ratio} = \frac{\text{ml sample} + \text{ml water} + 1\ (\text{ml acid})}{\text{ml sample}}$$

B For extracted sample refer to the extracted standard calibration curve.

mg/l = (mg/l in extract) × C

$$C = \text{concentration ratio} = \frac{\text{ml solvent}}{\text{ml sample}}$$

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This outline was prepared by E. F. McFarren, Chief, Analytical Quality Control, Bureau of Water Hygiene, EPA, 5555 Ridge Avenue, Cincinnati, OH 45213.

Descriptors: Absorption, Chemical Analysis, Instrumentation, Spectrophotometry, Water Analysis

ATOMIC ABSORPTION LABORATORY

LEAD ANALYSIS

Preparation of Standards:

A stock standard solution containing 1000 mg/l lead was prepared by dissolving 1.5984 g of Pb $(NO_3)_2$ in one liter of distilled deionized water.

From the stock solution, make up 200 ml standards containing 0.025, 0.050, 0.075, and 0.100 mg/l Pb.

Operating Conditions for P-E 303:

Wavelength:	217.
Range:	UV.
Slit:	4.
Source:	10 ma, hollow cathode.
Burner:	P-E premix.
Air:	20 psi. (30 psi. on tank)
Auxiliary Air:	9.0 on flowmeter
Acetylene:	8 psi.; 7.0 on flowmeter.
Flame:	clear and non-luminescent.
Sample Uptake Rate:	2-5 ml/min.

Procedure:

A Extraction of samples and standards:

1 Extract 200 ml aliquots of standards, unknown sample, control sample B, and a blank consisting of distilled water, according to step IV-D of the General Procedure.

B Setting up Instrument:

1 Insert proper hollow cathode tube in instrument.

2 Set operating conditions as specified for wavelength, range, slit, and source current.

3 Turn on the air supply and adjust tank regulator to 40 psi. Set air pressure on Burner Regulator to 20 psi. with auxiliary air valve closed. The air rotameter should indicate about 4.5.

4 Turn on the acetylene supply and adjust tank regulator to 8.0 psi. Adjust the fuel control valve to indicate 4.5 on the fuel rotameter.

5 Ignite the flame.

6 Adjust the auxiliary air needle valve to indicate 9.0 on the air rotameter and the fuel needle valve to indicate 7.0 on the fuel rotameter.

7 Make fine adjustments as required to produce a clear, non-luminescent flame while aspirating the solvent blank.

8 Zero the instrument while aspirating the extracted blank.

C Preparation of Standard Curve:

1 Aspirate the extracted standards and record % absorption.

2 Convert % absorption readings to absorbance by use of Table 1.

3 Plot absorbance readings vs. mg/l Pb.

D Running of Control Samples:

1 Do six determinations on the extracts of control sample A supplied by the instructor. After conversion of % absorption readings to absorbance, determine the concentration of lead in each control sample from the standard curve.

2 Calculate precision and accuracy of the six determinations.

3 Construct a control chart.

E Running of Unknown:

1 Aspirate extracted control sample B and extracted unknown sample and determine if readings are in control using the control chart.

2 If in control, record the concentration of the unknown sample after correcting for any dilution or concentration of the sample. For the calculation, refer to step V of the General Procedure.

This outline was prepared by Earl F. McFarren, Chief, Analytical Quality Control, Bureau of Water Hygiene, EPA, 5555 Ridge Avenue, Cincinnati, OH 45213.

Descriptors: Absorption, Chemical Analysis, Instrumentation, Laboratory Tests, Spectrophotometry, Water Analysis

ATOMIC ABSORPTION LABORATORY WORK SHEET

LEAD ANALYSIS

mg/l Pb	% Absorption	Absorbance
0.025	________	________
0.050	________	________
0.075	________	________
0.100	________	________
Control sample A	________	________
	________	________
	________	________
	________	________
	________	________
	________	________

Mean = ______ mg/l Pb   Standard deviation = ____

Control sample B ________

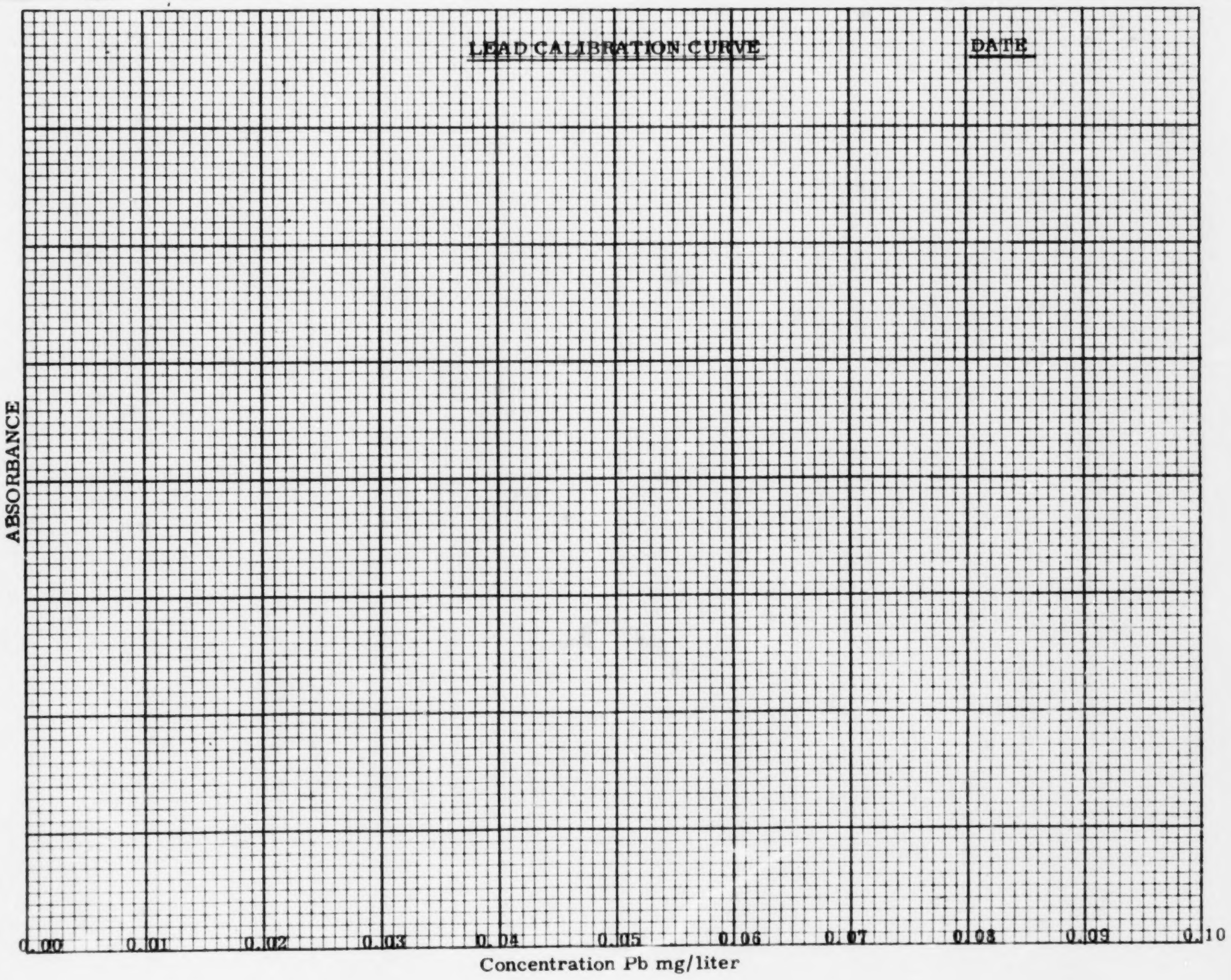
Concentration of control sample B = ________ mg/l Pb

Unknown ________ ________

Concentration of unknown = ______ mg/l Pb

Scale expansion = ________

Suppression (meter response) = ________



LEAD CALIBRATION CURVE

DATE

Laboratory Procedure For The Determination of Lead By Atomic Absorption - Extraction Method

I INTRODUCTION

When the concentration of a metal is not sufficiently high to determine directly, or when considerable dissolved solids are present in the sample, lead and other metals may be chelated and extracted with organic solvents. In this procedure, ammonium pyrrolidine dithiocarbanate (APDC) is the chelating agent, and methylisobutyl ketone (MIBK) is the organic solvent.

II REAGENTS

A Hydrochloric Acid, 0.3N
Dilute 25 ml of concentrated HCl to 1 liter with deionized water.

B Sodium Hydroxide, 2.5N
Dissolve 10g. of NaOH in deionized water and dilute to 100 ml.

C Bromphenol Blue Indicator
Dissolve 0.1g. of the solid in 100 ml. of 50% ethanol.

D APDC
Dissolve 1g. of the solid in deionized water. Dilute to 100 ml. and filter. Prepare fresh just before use.

E MIBK

F Lead Nitrate Stock Standard Solution
(1 mg Pb^{+2} / ml)
Dissolve 1.599 g. of analytical reagent grade Pb(NO$_3$)$_2$ in deionized water. Add 10 ml of concentrated HNO$_3$ and dilute to 1 liter.

G Lead Nitrate Working Standard
(10.0 mg Pb^{+2}/1)
Dilute 10.0 ml of the stock standard to 1 liter with deionized water.

III PROCEDURE

A Calibration Standards

Using 200 ml volumetric flasks, prepare five solutions (see following table) for the calibration graph. Use a 10 ml graduated pipette for measuring the lead nitrate working standard and a 100 ml graduate cylinder for measuring the deionized water.

ml of Lead Nitrate Working Std. Sol.	ml of Deionized Water
0.0	100.0
2.5	97.5
5.0	95.0
7.5	92.5
10.0	90.0

Total ml	Pb^{+2} conc. (mg/l) in the MIBK
100.0	0.0
100.0	2.5
100.0	5.0
100.0	7.5
100.0	10.0

B Pipette some accurately known volume of sample between 3.0 and 7.0 ml into a sixth volumetric flask and add sufficient deionized water to bring the final volume to 100 ml.

C Using an eyedropper, add 2 drops of bromphenol blue indicator to each of the six flasks.

D Using an eyedropper, add 2.5 N NaOH to each flask until a blue color persists. About 1 drop will suffice.

E Using an eyedropper, add 0.3 N HCl dropwise until the blue color just disappears. About 7 drops will suffice.

F Pipette 2.0 ml of 0.3 N HCl into each flask.

G Pipette into each flask 2.5 ml of the APDC solution. Mix well.

H Pipette 10 ml MIBK into the six flasks.

I Shake all flasks vigorously for one minute.

J Add sufficient deionized water to each flask so that the top of the MIBK layer is within about one inch of the top of the volumetric flask.

K Allow the two layers to separate completely.

L Aspirate the MIBK layer from each flask into the Model IL-153 atomic absorption instrument and record the scale readings.

IV CALIBRATION GRAPH

A Plot the scale readings vs. the Pb^{+2} conc. in the MIBK layer (see table) for the five calibration standards.

B From the scale reading and calibration graph, determine the conc. of Pb^{+2} in the sample MIBK layer.

V REFERENCE

Methods for Chemical Analysis of Water and Wastes, 1971, Environmental Protection Agency, Water Quality Office, Analytical Quality Control Laboratory, Cincinnati, OH.

This outline was prepared by Charles R. Feldmann, Chemist, National Training Ctr., EPA, WPO, Cincinnati, OH 45268.

Descriptors: Chemical Analysis, Heavy Metals, Laboratory Tests, Lead, Metals, Water Analysis

ANALYSIS OF WATER SAMPLE FOR CHLORINATED HYDROCARBONS

I INTRODUCTION

It is the purpose of this laboratory to acquaint the trainee with techniques for extraction of pesticides from water followed by identification in the Gas Chromatograph.

II EQUIPMENT

A Glass Ware

1 1000 ml separatory flask

2 Kuderna-Danish extractor

3 Funnel

4 Glass wool

5 Medicine dropper

6 Spotting capillary

7 10 ml volumetric flask

8 25 ml graduate flask

B Chemicals

1 Hexane

2 Sodium sulfate

III EXTRACTION PROCEDURE

A Hexane Extraction

1 Add twenty-five ml (25 ml) hexane to 1000 ml separatory flask containing 500 ml water sample. Flask is rocked back and forth gently and shaken lightly for 2 minutes. CAUTION: Release pressure through stopcock.

2 Remove the stopper from the separatory funnel and drain the water sample through the stopcock into a liter beaker. The hexane is poured off the top into an Erlenmeyer flask.

3 The water sample in the beaker is poured back into the separatory flask and another 25 ml portion of hexane is added. Flask is rocked back and forth gently and shaken lightly for 2 minutes. The water sample is drained through the stopcock into a liter beaker. The hexane is poured off the top into an Erlenmeyer flask.

4 Repeat step 3 two more times.

B Drying of Hexane Extract

1 Place a tuft of glass wool in bottom of funnel. Pour granulated sodium sulfate (anhydrous), the Na_2SO_4 filling approximately 2/3 of the funnel volume.

2 Position Kuderna-Danish evaporator on ring stand so that the stem of the funnel containing Na_2SO_4 is well within the neck of the evaporator.

3 Check fitting of collection tube attached to evaporator by two springs or small rubber bands.

4 Pour the four 25 ml (100 ml vol) hexane extractions through the Na_2SO_4 into the evaporator.

5 Rinse Erlenmeyer flask and Na_2SO_4 three times with approximately 10 ml portions of hexane.

6 Place condenser into the Kuderna-Danish evaporator and take to steam bath.

7 Place evaporator in clamp above steam bath opening.

8 Turn steam up slowly until hexane begins to boil.

9 Evaporation should be adjusted so that the hexane vapors are rushing through condenser.

10 When hexane has evaporated to a few mls in the collector tube, turn off steam and remove the Kuderna-Danish evaporator from the steam bath.

11 Carefully wipe the outside of the Kuderna - Danish evaporator so as to remove all traces of condensed water.

C Preparation of Sample for Gas Chromatography

1 Place a small tuft of glass wool into the tip of a medicine dropper, and fill 2/3 with Na_2SO_4. It is necessary to have an inch of empty space at top of dropper.

2 Place a paper clip on the dropper and set the dropper in the neck of 10 ml volumetric flask.

3 With another medicine dropper transfer the contents of collector tube into medicine dropper.

4 Using the capillary as a stirring rod pour the last few drops of extract down the capillary into flask. Rinse collector tube and micro Na_2SO_4 column with two dropperfuls of hexane.

5 Bring volume to 10 ml with hexane. Identify volumetric flask with a circle tag.

IV DETERMINATIVE STEP

A Prepare a standard curve on the electron capture gas chromatograph for lindane, aldrin, and heptachlor epoxide.

B Inject 5-10 μl of this extract into the electron capture gas chromatograph. Compute the concentrations of the pesticides found in μg/l.

1 $$\frac{\text{ng pesticide found}}{\mu\text{l hexane injected}} \times \frac{1000\ \mu\text{l}}{\text{ml}} \times \frac{0.001\ \mu\text{g}}{\text{ng}} = \frac{\mu\text{g pesticide found}}{\text{ml hexane injected}}$$

2 $$\frac{\mu\text{g pesticide found}}{\text{ml hexane injected}}\ (\text{from } 1) \times 10\ \text{ml} =$$

μg of pesticide in the 10 ml volumetric flask

3 μg of pesticide (from 2) x 2 = μg of pesticide/liter of sample

2 is a factor introduced because the answer is desired on a per liter basis, and the original sample volume was 500 ml.

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This outline was prepared by Betty A. Punghorst, Former Chemist, National Training Center, EPA, WPO, Cincinnati, OH 45268, and revised by Charles R. Feldmann, Chemist, National Training Ctr.

Descriptors: Aldrin, Chemical Analysis, Chlorinated Hydrocarbon Pesticides, Gas Chromatography, Laboratory Tests, Pesticide Residues, Pesticides, Water Analysis

ANALYSIS OF SOIL SAMPLE FOR CHLORINATED HYDROCARBON AND ORGANOPHOSPHORUS COMPOUNDS

I OBJECTIVE

To enable the trainee to identify and quantitate chlorinated hydrocarbon and organophosphorus compounds in a silt loam or red clay sample using soxhlet extraction, florisil column chromatography, thin layer chromatography, anticholinesterase activity measurement and microcoulometric gas chromatography.

II SOXHLET EXTRACTION AND COLUMN CHROMATOGRAPHY

A Reagents

1 Solvents

a Hexane, redistilled in glass BP 68° - 69°C

b Petroleum ether, redistilled in glass, BP 30-60°C

c Acetone and ether, ACS grade

d Eluting mixture, 20 parts ethyl ether and 80 parts petroleum ether.

2 Extraction thimbles, Whatman single thickness, 43 x 123 mm.

3 Florisil 60-100 mesh

Florisil is furnished in 3 activation grades: 230°F (110°C), 500°F (260°C), and 1200°F (650°C). Suitability activated product has been heated at 650°C not less than 1 hour nor more than 3 hours. The two lower activation grades must be heated 1 - 3 hours at 650°C in a preheated muffle cooled in an oven at 130°C, transferred to glass-stoppered flasks or bottles and stored in a desiccator. Heat at 130°C not less than 5 hours (overnight is convenient if not used in 10 days). Florisil that has been overheated at 650°C cannot be treated in any way to restore adsorbent properties for this determination.

For further notes on standardization procedures for florisil see reference 1 (Section 2.12).

B Special Equipment Required

1 Soxhlet Extractor, flask capacity (500 ml), with Allihn condenser.

2 Chromatographic tube (20 x 400 mm) with Ultramax valve.

3 Thin layer chromatography equipment.

4 Microcoulometric gas chromatograph equipped with sulfur and chloride cells.

C Preparation of Sample

1 Allow the soil sample to dry thoroughly at room temperature for about a week. This is best accomplished by breaking up the large particles and spreading out in a glass dish.

2 Grind up sample with a mortar and pestle and pass through a #30 or #40 sieve.

3 Select a 100 gram aliquot and place in a pre-extracted thimble. Place a small wad of pre-extracted glass wool on top of the soil.

D Soxhlet Extraction

1 Using 300 ml 10% acetone in hexane, extract soil sample for 24 hours (at 60°C).

2 Transfer extract to 400 ml beaker and concentrate on steam bath (or in Kuderna-Danish evaporator) to about 10 ml.

E Column Chromatography

1 With stirring rod, insert a small tuft of glass wool into the chromatographic column (to act as a mat for the adsorbent).

To column add 4-1/2 - 5 inches of florisil which has been activated overnight at 130°C and stored in a desiccator to cool. Tamp column to 4 inches using a rubber stopper.

2 Place a 50 ml graduated cylinder under the column. Carefully pour 50 ml of petroleum ether into the column; do not allow the petroleum ether to splash onto the florisil, but pour it down the sides of the column. Adjust the stopcock of the column so as to maintain an elution rate of 1 - 2 drops/sec. The function of the petroleum ether is to prewet the florisil. The stopcock will have to be periodically adjusted so as to maintain the elution rate.

3 While the petroleum ether is moving through the column, add 10 ml of petroleum ether to the small beaker containing the soil residue. Use a glass rod to thoroughly mix the residue and ether.

4 When the meniscus of the 50 ml portion just reaches the surface of the florisil, replace the 50 ml graduated cylinder under the column with a 200 ml volumetric flask. Immediately add the 10 ml of petroleum ether plus soil residue to the column. Rinse the small beaker three times with 2-3 ml portions of petroleum ether. Add the rinsings to the column. Leave the column stopcock open.

5 When the meniscus of the 10 ml of petroleum ether plus rinsings just reaches the surface of the florisil, begin adding to the column 200 ml of a solution containing 20% ethyl ether in petroleum ether. Throughout the addition of the 200 ml maintain the 1 - 2 drop/sec. elution rate. During elution column may tend to separate. If this occurs, retramp with the rubber stopper. The florisil column does not retain certain pigments present in soil. Consequently, the eluate may have a yellow appearance.

6 After the elution is completed, bring the final volume in the volumetric flask to 200 ml with petroleum ether. Pipette a 10 ml aliquot into a Kuderna-Danish cup; stopper, and label for microcoulometric gas chromatography (MCGC).

F Evaporation of Eluate

1 Transfer remaining eluate (190 ml) to a clean Kuderna-Danish evaporator. Evaporate to 2.0 ml. Evaporate further with clean, filtered air to 0.2 ml. Dilute back to 1.0 ml with hexane.

2 Stopper and label for thin layer chromatography (TLC).

REFERENCES

Barry, H.C., et. al. Pesticide Analytical Manual (Vol. 1). U.S. Dept. of Health, Education and Welfare, Food and Drug Administration, July 1965.

This outline was prepared by Betty Ann Punghorst, Former Chemist, National Training Center, EPA, WPO, Cincinnati, OH 45268, and revised by Charles R. Feldmann, Chemist, National Training Center.

Descriptors: Chemical Analysis, Chlorinated Hydrocarbon Pesticides, Gas Chromatography, Laboratory Tests, Organophosphorus Pesticides, Pesticide Residues, Pesticides, Soil Analysis

ANALYSIS OF SOIL SAMPLE FOR CHLORINATED HYDROCARBON AND ORGANOPHOSPHORUS COMPOUNDS

I THIN-LAYER CHROMATOGRAPHY LABORATORY FOR SEPARATION OF CHLORINATED HYDROCARBON PESTICIDE RESIDUES

A Procedure

1 Preparation of plates

a Weigh a 40 gram aliquot of Al_2O_3 in a 500 ml centrifuge bottle.

b Add 80 ml 0.03 $\underline{N}$ HNO_3. Shake well and centrifuge at 1200 rpm for 1-2 minutes.

c Decant the supernatant liquid into a 100 ml graduated cylinder, and record the volume (35-40 ml should be recovered).

d Add 80 ml distilled H_2O, break up cake on the bottom of the bottle with a glass rod, shake well and centrifuge as before.

e Decant and record the volume of the supernatant liquid recovered (60-70 ml).

f Repeat the washing step with two additional 80 ml portions of H_2O, centrifuge and decant as before.

g Weigh the Al_2O_3 and H_2O which has been retained (approx. 100 g).

h Add 10 ml 1.0% $AgNO_3$ and enough H_2O to make the total weight 120 g.

i Shake well, place in applicator and prepare plates.

j Let plates dry on mounting board for approximately 15 minutes.

k Place in drying rack in a vertical position at 100°C for 30 minutes.

l Remove the plates and store in a desiccator.

B Separation of Pesticides by Thin-Layer Chromatography

1 Spotting plate

a Position of plate

Scrape 1/4 inch area on two opposite sides of plate. Lay a kimwipe open on top of bench and place an aluminum oxide coated plate on kimwipe.

b Place a kimwipe folded 1/4 size on the left hand side of the work area.

c Place 4 sample vials, unknown, DDT (1μg/μl), DDE (1μg/μl) and dieldrin (1μg/μl) along side of folded kimwipe.

d Insert (5 μl) capillary spotting tube in small beaker of hexane and touch to folded kimwipe until all hexane has been removed from capillary. Repeat this five times.

e Place template with graduated edge upon the aluminum oxide $AgNO_3$ impregnated plate with starting line about one (1) inch from bottom.

f Mark this position with pencil on both sides of plate. Also mark 10 cm line.

g Rinse capillary twice with DDT removing contents each time on kimwipe.

h At spotting position of template, touch capillary filled with DDT once lightly on plate removing approximately 1/4 of contents of capillary (plate position #1).

i At position #2 spot two times putting 1/2 the contents of the capillary on the plate. Permit each spot to dry before respotting.

j Remove contents of the capillary on kimwipe and refill from vial.

k At position #3 spot the contents of the capillary (5 μl) by lightly touching the capillary to the plate removing about 1/4 of the contents with each spotting. Permit the spots to dry before re-spotting.

l Rinse capillary 5 times in hexane.

m At positions #4 - #6, spot DDE; repeat spotting procedure as described in items h-1.

n At positions #7 - #9, spot dieldrin; repeat spotting procedure as described in items h-1.

o At positions #10 - #12, spot soil extract; repeat spotting procedure as described in items h-1.

2 Development in Solvent

a Place plate, coated surface facing solvent saturated paper, in developing tank containing 1% ethyl ether in hexane with marked spotting line down; develop to 10 cm line.

b Lift off top of jar and remove plate, lifting plate with finger tips of both hands. Dry in air for 10 minutes.

c Place in ultraviolet light box and expose for 15 minutes.

d Measure the distance of centers of the spots from starting line and compute the R_f values. Record data on data sheet.

$$R_f \text{ value} = \frac{\text{distance in cms of pesticide from start}}{\text{distance in cms of solvent front from start}}$$

REFERENCES

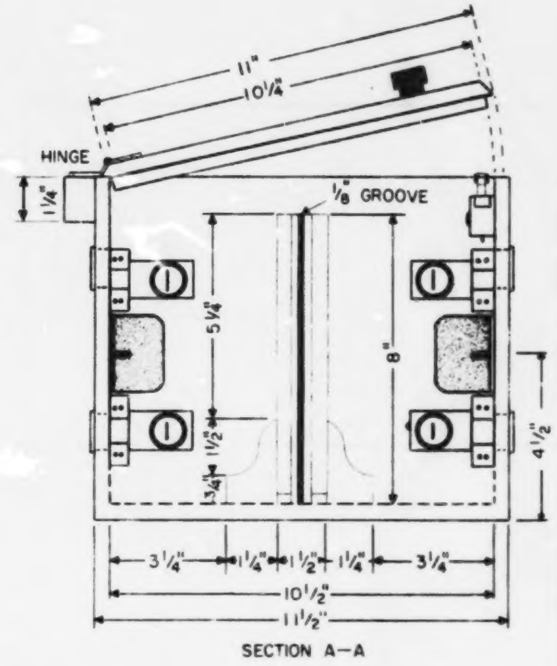
1 Barry, H.C., et.al. Pesticide Analytical Manual (Vol. I). U.S. Department of Health, Education and Welfare, Food and Drug Administration, July 1965.

2 Breidenbach, A.W. The Identification and Measurement of Chlorinated Hydrocarbon Pesticides in Surface Waters. U.S. Department of the Interior, Publication No. WP-22 (1966).

This outline was prepared by Betty Ann Punghorst, formerly Chemist, National Training Center, Cincinnati, OH 45268

THIN-LAYER CHROMATOGRAPHY DATA SHEET

Sample	Solvent	Solvent Front Distance from Start (cm)	Sample Position Distance from Start (cm)	R_f	R_x



SECTION A—A

NOTE.
SEE FOLLOWING SUPPLEMENTAL
SHEET NO.1 FOR CUTWAY VIEW

LEGEND

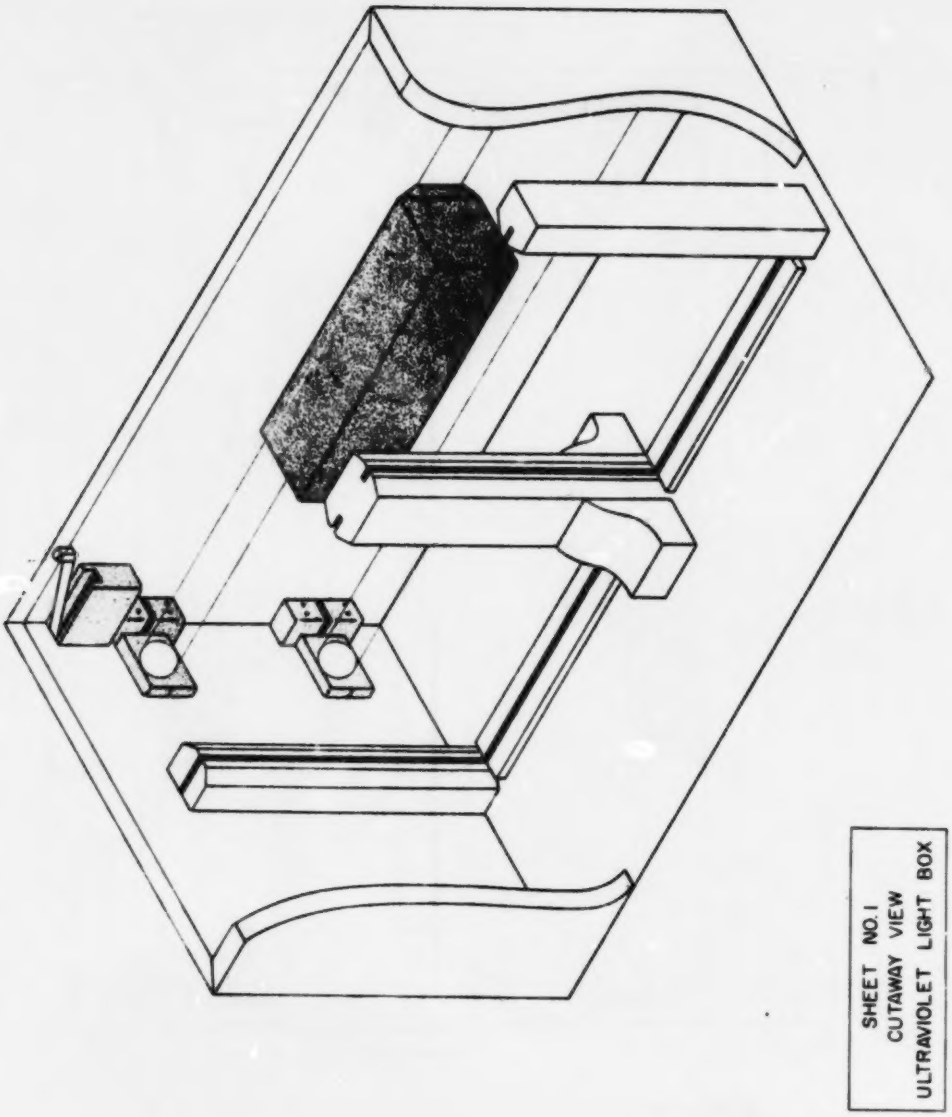
NO. NEEDED	ITEM
4 EACH	— FLUORESENT LAMPHOLDER BYRANT #4300 OR EQUIV.
4 EACH	— GERMICIDAL LAMPS, 15 WATT GE # G15T8 OR EQUIV.
1 EACH	— TOGGLE SWITCH, SINGLE POLE, 10 AMP.
1 EACH	— MICRO SWITCH, TYPE W, 10-15 AMP.
2 EACH	— BALLAST, TRIGGER START, GE #6G3512 OR EQUIV.
4 EACH	— 1" ALUMINUM VENTILATING LOUVERS.
1 PAIR	— SMALL CABINET HINGES.
1 EACH	— CABINET PULL.
1 EACH	— 6 FT. LINE CORD

ULTRAVIOLET LIGHT BOX

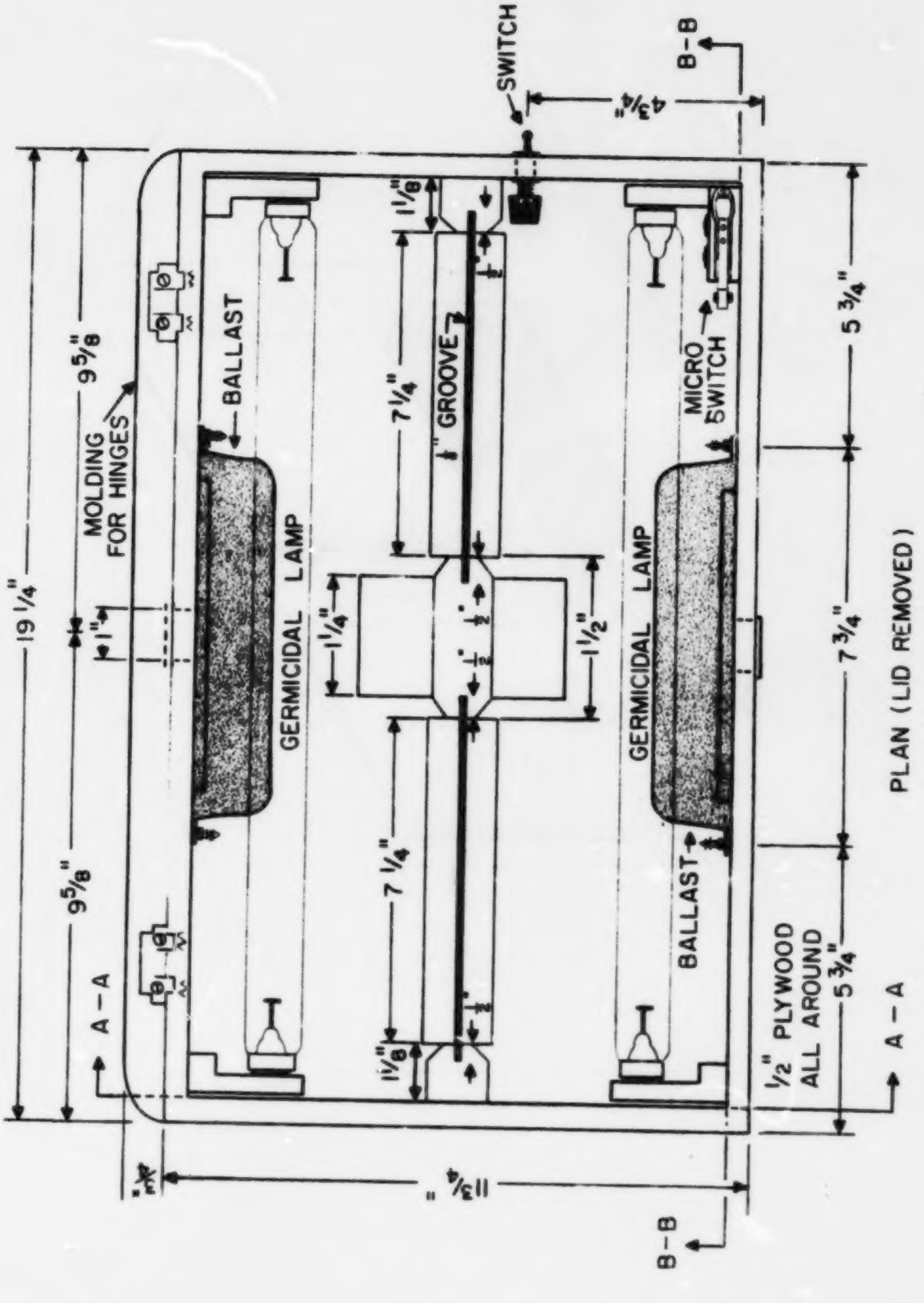
DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
U.S. Public Health Service
ROBERT A. TAFT
SANITARY ENGINEERING CENTER

DESIGNED BY H.L.Feig
DRAWN BY: J.O.Boyer. From original pilot model
Pilot model constructed in ROBERT A. TAFT SANITARY ENGINEERING CENTER model shop supervised by J. Costeit

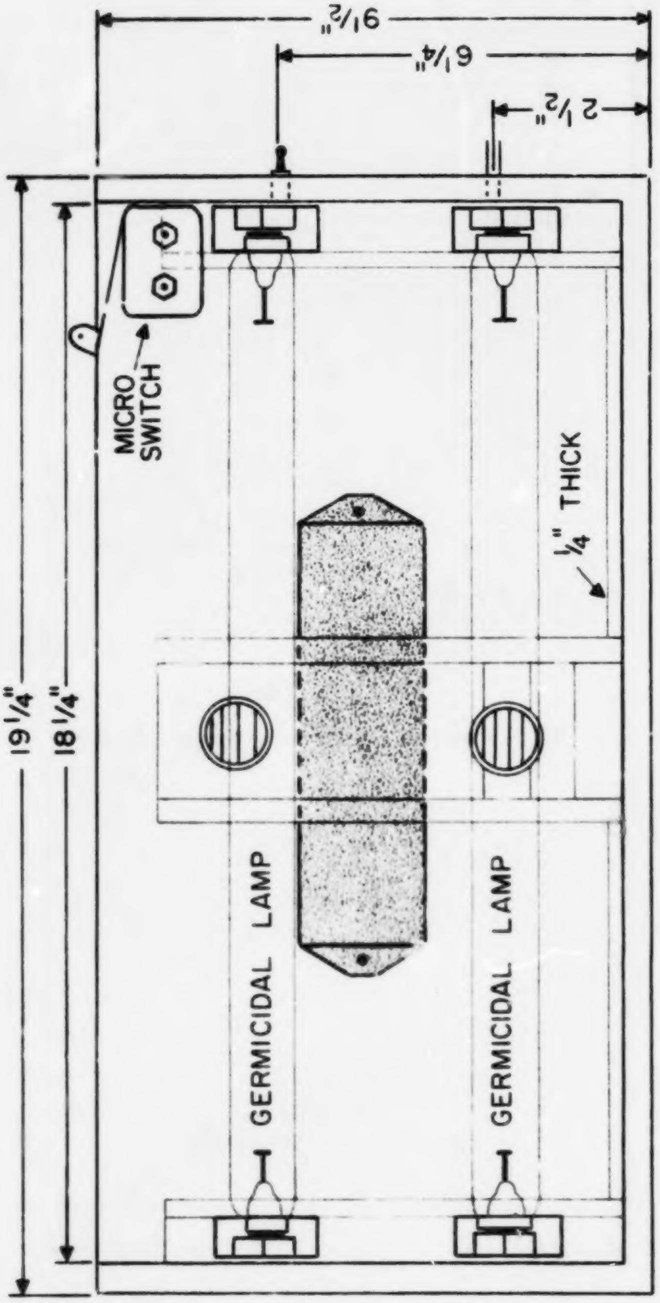
SCALE Date Jan 9, 1961



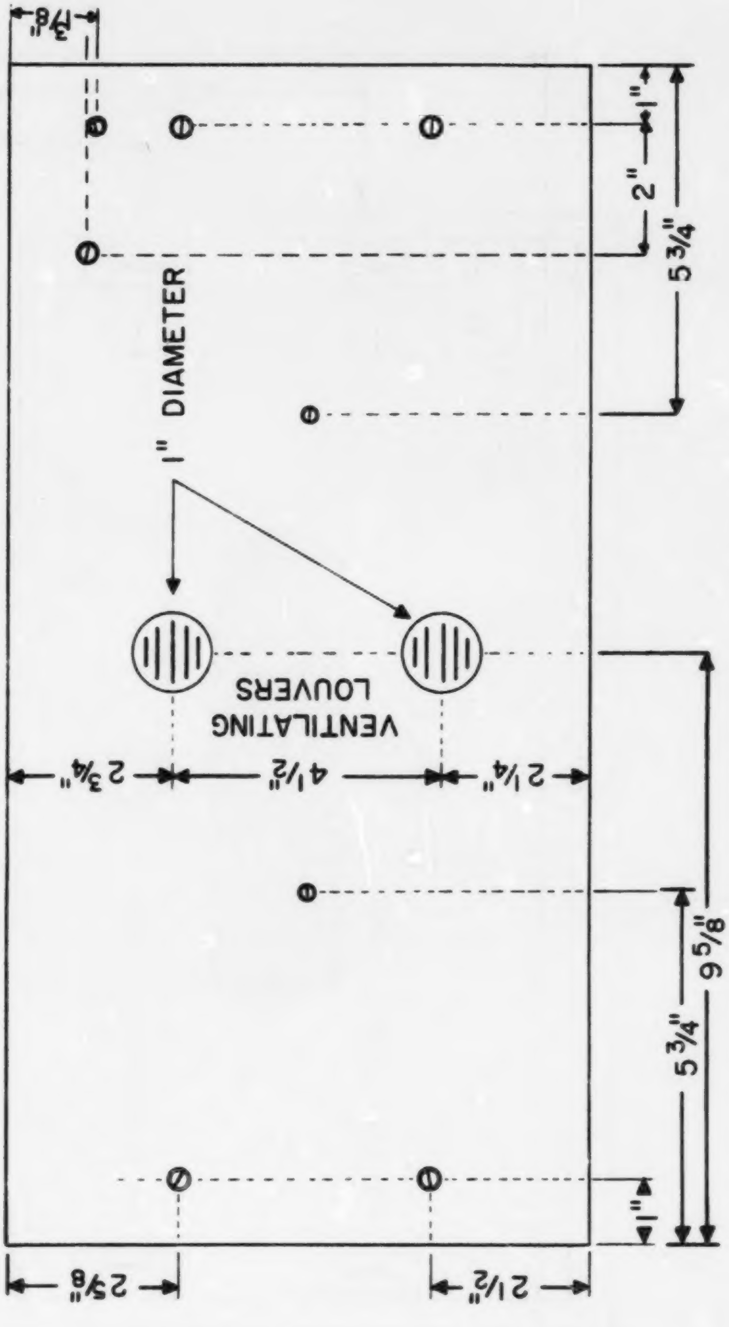
SHEET NO. 1
CUTAWAY VIEW
ULTRAVIOLET LIGHT BOX


SWITCH
B-B
4 3/4"
19 1/4"
9 5/8"
MOLDING FOR HINGES
BALLAST
1 1/8"
7 1/4"
1/4" GROOVE
MICRO SWITCH
5 3/4"
GERMICIDAL LAMP
1"
1 1/4"
1/2"
1 1/2"
7 3/4"
GERMICIDAL LAMP
9 5/8"
7 1/4"
BALLAST
A-A
1 1/8"
1/2" PLYWOOD ALL AROUND
5 3/4"
A-A
3/4"
11 3/4"
B-B

PLAN (LID REMOVED)


9 1/2"
6 1/4"
2 1/2"
19 1/4"
18 1/4"
MICRO SWITCH
1/4" THICK
GERMICIDAL LAMP
GERMICIDAL LAMP

SECTION B — B


1 7/8"
1"
2"
5 3/4"
1" DIAMETER
VENTILATING LOUVERS
2 3/4"
4 1/2"
2 1/4"
5 3/4"
9 5/8"
1"
2 5/8"
2 1/2"

FRONT ELEVATION

ANALYSIS OF SOIL SAMPLE FOR CHLORINATED HYDROCARBON AND ORGANOPHOSPHORUS COMPOUNDS

I A MODIFIED KOVAC'S THIN-LAYER CHROMATOGRAPHY FOR ORGANO THIOPHOSPHATE PESTICIDES

A Reagents

1 Adsorbents

Aluminum oxide G

2 Solvent systems

a Immobile

15% - 20% N, N-dimethyl formamide in ethyl ether

b Mobile

Methyl-cyclohexane

3 Chromogenic agents

a Stock dye solution

1 g tetrabromophenolphthalein ethyl ester in 100 ml acetone

b Working dye solution

Dilute 10 ml stock dye solution in 50 ml acetone.

c AgNO$_3$ solution

Dissolve 0.5 g AgNO$_3$ in 25 ml distilled water and dilute to 100 ml with acetone.

d Citric acid solution

Dissolve 5 g citric acid in 50 ml distilled water, dilute to 100 ml with acetone.

B Pesticide Samples

1 1 μg/μl solutions of following pesticides in n-hexane: ronnel, parathion, systox, dipterex, malathion.

2 Combination of two pesticides in one sample 0.5 μg/μl of each: ronnel, parathion.

3 Extract of mud sample containing parathion.

C Equipment

1 Glass plates

8 x 8" double strength coated with 250μ thickness of aluminum oxide G.

2 2 μl capillary and spotting tube

3 50 ml beaker containing n-hexane

4 Developing tank

5 Spray flask or canister

a Tetrabromophenolphthalein ethyl ester dye solution

b AgNO$_3$ solution

c Citric acid solution

d N, N dimethyl formamide in ethyl ether

D Procedure

1 Spotting plate

a Position plate

Scrape 1/4 inch area on two opposite sides of plate. Lay a kimwipe open on top of bench and place an aluminum oxide coated plate on kimwipe.

b Place a folded kimwipe on the left hand side of plate.

c Place 6 sample vials alongside of folded kimwipe.

1) Ronnel

2) Parathion

3) Systox

4) Dipterex

5) Malathion

6) Combination sample

d Insert (2 μl) capillary spotting tube in small beaker of hexane and touch to folded kimwipe until all hexane has been removed from capillary. Repeat this five times.

e Place template with graduated edge upon the plate with starting line about one (1) inch from bottom.

f Mark this position as spotting line and 10 cm above this line mark the coated surface at the side of the plate.

g Rinse capillary twice with the ronnel solution.

h At spotting position #1 of template, touch capillary filled with ronnel once lightly on plate removing approximately 1/4 of contents of capillary. Permit spot to dry then continue the spotting technique until all of the 2 μl have been applied at the same spot.

i Similarly spot the other pesticides parathion, systox, dipterex, malathion, the combination sample and the soil extract at the next six (6) positions indicated by the graduations on the template. Place 15 ml centrifuge tube containing soil extract aside and mark for determination of anti-cholinesterase activity.

j Rinse capillary 5 times in hexane between each pesticide spotting.

2 Development in Solvent

a Place plate in hood against spraying board with coated surface facing toward front of hood.

b Spray moderately with N, N dimethyl formamide-ethyl ether solution.

c Place plate spotting line down and coated surface facing solvent saturated paper, in developing tank containing methyl-cyclohexane.

d Develop to the mark, and remove plate to hood and place against spraying board.

3 Chromogenic Development

a Immediately spray with tetrabromophenolphthalein ethyl ester solution.

b Spray with $AgNO_3$ solution and let stand for 2 minutes.

c Spray with citric acid solution.

d Measure the distance of centers of the spots to starting line and compute the R_f values.

$$R_f = \frac{\text{distance in cms of pesticide from start}}{\text{distance in cms of solvent front from start}}$$

REFERENCES

Kovacs, M.F. Thin-Layer Chromatography for Organo Thiophosphate Pesticide Residue Determination, JAOAC, Vol. 47, No. 6, 1097 (1964).

Descriptors: Adsorption, Chemical Analysis, Chlorinated Hydrocarbon Pesticides, Chromatography, Laboratory Tests, Organophosphorus Pesticides, Pesticide Residues, Pesticides, Soil Analysis

ANALYSIS OF SOIL SAMPLE FOR CHLORINATED HYDROCARBON AND ORGANOPHOSPHORUS COMPOUNDS

I MICROCOULOMETRIC GAS CHROMATOGRAPHY

A Description of Instrumentation

1 Detector

Dohrmann Model C-200 microcoulometer equipped with T-300 titration cells.

2 Operating conditions

a Column conditions

1) Temperature ________°C

2) Flow rate ________ml/min

3) Diameter ________inches

4) Length ________feet

5) Solid support ________

6) Stationary phase ________

b Combustion furnace

1) Furnace temperature ________°C

2) Inlet temperature ________°C

3) O_2 flow rate ________cc/min

4) N_2 sweep rate ________cc/min

c Titration cell

1) Type ________

2) Mode ________

3) Range Ohms ________

4) Bias Volts ________

5) Electrolyte ________

B Background Theory

1 Coulometric titration

a Chloride cell

The microcoulometric titration cell (chloride) used as a gas chromatographic detector has for its basis the coulometric titration of chloride ions with silver ions. Chloride ions are produced when the effluent from the gas chromatographic column enters the combustion furnace and any chlorinated hydrocarbons present are broken down into HCl, CO_2 and H_2O.

1) When chloride ions enter the titration cell, the deficiency of silver ions is registered by a reference/sensor electrode pair (see Figure 1).

2) The voltage difference between the reference/sensor pair and an applied bias voltage is amplified and fed as an input to the generator circuit.

3) A subsequent regeneration of silver ions occurs from the generator anode-cathode pair.

4) During the course of regeneration of titrant, the generation current passes through a series of resistors.

5) The voltage drop across these resistors is fed as the input to the recorder. The area of the peak produced is a measure of the coulombs consumed in the regeneration of titrant, which in turn is a measure of the chloride entering the cell.

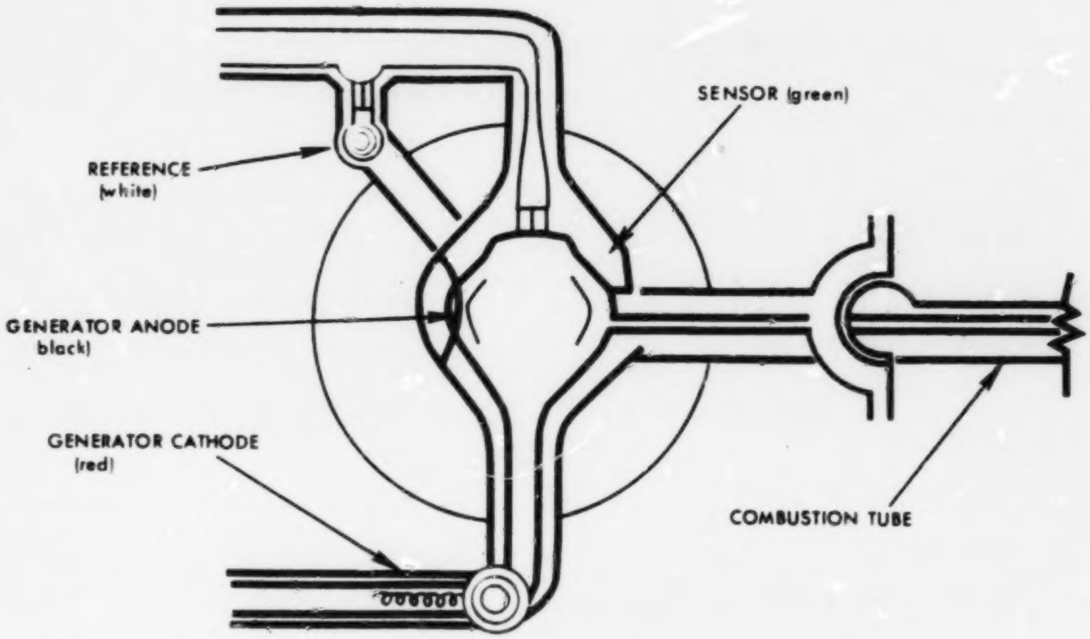


FIGURE 1
MICROCOULOMETRIC TITRATION CELL
(LOW GAIN MODE)

b Sulfur cell

This cell operates on the same principles as the chloride cell except that sulfur containing compounds produce SO_2 and H_2O in the combustion furnace. The SO_2 produced is coulometrically titrated with tri-iodide ion in the titration cell.

C Chloride containing Compounds (Chloride Cell)

1 Set electrodes in low gain position.

2 Set GAIN switch to LO (This supplies a gain of approximately 200 to the coulometer amplifier).

3 Set FUNCTION switch to BIAS READ and adjust BIAS MILLIVOLTS with BIAS SET knob to 12 divisions to the left of zero. This supplies 240 bias millivolts to the cell.

4 Adjust stirring rate in titration cell to slowest position.

5 Set FUNCTION switch to GENERATOR READ. Generator volt meter should read 0 or to the left of 0. If the meter reads to the right of 0, add fresh electrolyte to the cell and flush through both stopcocks with electrolyte (70% acetic acid) until the meter reads zero or to the left of zero. (NOTE: Final level of electrolyte should be at black mark on cell).

6 Turn recorder power and chart on and adjust needle on recorder to zero with RECORDER ZERO knob on coulometer amplifier (NOTE: Needle on recorder is at 0 when integrator draws a straight line).

7 Set FUNCTION switch to OPERATE. Set RANGE OHMS to 150 (150 ohms) and wait until cell generates enough titrant to reach null.

8 With VENT open, inject 50 NG of dieldrin into the gas chromatograph. Depress MARK button.

9 After one minute close the vent and wait for dieldrin peak. Measure and record the peak height and peak area.

10 With VENT open inject 50 μl of sample eluate from florisil column. After one minute close the vent and wait for peak. Measure peak height and peak area and calculate μg/gm of dieldrin in soil sample.

D Sulfur Containing Compounds (Sulfur Cell)

1 Set electrodes in low gain position.

2 Set GAIN switch to LO. (This supplies a gain of approximately 200 to the coulometer amplifier).

3 Set FUNCTION switch to BIAS READ and adjust BIAS MILLIVOLTS with BIAS SET knob to 6 divisions to the left of zero. This supplies 720 bias millivolts to the cell.

4 Adjust stirring rate in titration cell to slowest position.

5 Set FUNCTION switch to GENERATOR READ. Generator volt meter should read 0 or to the left of 0. If the meter reads to the right of 0, add fresh electrolyte to the cell and flush through both stopcocks with electrolyte (0.05% KI and 0.04% HOAC) until the meter reads zero or to the left of zero. (NOTE: Final level of electrolyte should be at black mark on cell).

6 Turn recorder power and chart on and adjust needle on recorder to zero with RECORDER ZERO knob on coulometer amplifier (NOTE: Needle on recorder is at 0 when integrator draws a straight line).

7 Set FUNCTION switch to OPERATE. Set RANGE OHMS to 100 (100 ohms) and wait until cell generates enough titrant to reach null.

8 With VENT open, inject 500 NG of malathion and parathion. Depress MARK button.

9 After one minute close the vent and wait for peaks. Measure and record the peak heights and peak areas.

10 Concentrate 10 ml of sample eluate from florisil column to 1 ml in 15 ml centrifuge tube. With VENT open, inject 50 μl of concentrated sample eluate. After one minute, close the vent and wait for peaks. Measure peak height and peak area and calculate μg/gm of pesticide in soil sample.

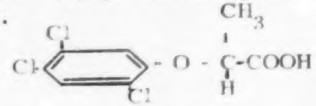
Descriptors: Chemical Analysis, Chlorinated Hydrocarbon Pesticides, Gas Chromatography, Laboratory Tests, Organophosphorus Pesticides, Pesticide Residues, Pesticides, Soil Analysis

ANALYSIS OF RAW WATER FOR SILVEX

(Rapid Screening Method)

I OBJECTIVE

To enable the trainee to apply to a raw water sample the technique of esterification in the analysis of the phenylalkanoic acid herbicide silvex 2-(2, 4, 5-trichlorophenoxy) propionic acid.



II REAGENTS

A 1:1 sulfuric acid

B Diethyl ether, anhydrous, reagent grade

C Sodium sulfate (checked for interfering ECGC peaks)

D Boron trifluoride - methanol solution (14% W/V) Available already prepared from Applied Science Laboratories.

E Hexane, redistilled in glass BP 68-69°C.

F Silvex standard (1 ml = 1 µ g Silvex in ethyl acetate)

III EXTRACTION

A Add 25 ml of raw water to 50 ml volumetric flask. Add enough sodium sulfate to saturate the water. Add 1 ml of 1:1 H_2SO_4 and exactly 5 ml of diethyl ether to the flask. Make to volume with saturated sodium sulfate solution and shake the contents vigorously for 1 minute (CAUTION: Ether pressure may cause loss of sample).

B Transfer 2 ml of the ether layer to a 10 ml volumetric flask. Evaporate the ether layer to dryness with air.

C Pipette 0.2, 0.5, and 1 ml of silvex standard (1 mg/1 into a series of 10 ml volumetric flasks. Evaporate with air and proceed with methylation.

IV METHYLATION

A Pipette 1 ml of the BF_3 - methanol solution into each of the sample and standard flasks. Hold the flasks in a boiling water bath for 2 minutes with frequent swirling and then cool.

B Pipette exactly 1 ml of hexane into each flask. Make to volume with 2% Na_2SO_4 solution and shake the contents vigorously for one minute. Allow to stand for at least an hour before proceeding with the determinative technique.

V DETERMINATIVE STEP

A Inject 5 µl of each standard(taken from the hexane layer)into a gas chromatograph equipped with an electron capture detector. Measure peak heights and prepare a standard curve.

B Inject 5 µl of the sample(taken from the hexane layer)into the gas chromatograph. Measure the peak height and calculate the amount of silvex in the sample.

1. $$\frac{\text{ng silvex found}}{\mu\text{l hexane injected}} \times \frac{1000\ \mu\text{l}}{\text{ml}} \times \frac{0.001\ \mu\text{g}}{\text{ng}} = \frac{\mu\text{g silvex found}}{\text{ml hexane injected}}$$

2. $$\frac{\mu\text{g}}{\text{ml}}\ (\text{from 1}) \times \frac{5}{2} \times 40 = \frac{\mu\text{g silvex}}{\text{l of raw water}}$$

5/2 is a factor introduced because only 2 ml of the diethyl ether were used.

40 is a factor introduced because the answer is desired on a per liter basis and the original sample volume was 25 ml.

VI NOTES ON PROCEDURE

A This is a screening method for silvex which should also be applicable to other phenylalkanoic acid herbicides. For more sensitive and comprehensive methods consult reference 1.

B This method measures both silvex and its esters. A bicarbonate extraction technique for separating the acid and its esters in the original sample and measuring them independently has been applied (see reference 3).

REFERENCES

1 Burchfield, H.P. and Johnson, D.E. Manual of Methods for the Analysis of Pesticide Residues. Section IV (Extraction and Cleanup of Anionic Pesticides). DHEW, PHS, October 1964.

2 Gutenmann, Walter H. and Lisk, Donald J. Gas Chromatographic Method for Silvex. JAWWA 56:189 (1964).

3 Organic Pesticides in the Environment. Advances in Chemistry Series (#60). Determination of Silvex and its Low Volatile Esters in Water and Muds (John D. Pope, Jr., W.S. Cox, III, and A.R. Grzenda). Washington, D.C. American Chemical Society. (1966).

This outline was prepared by Betty A. Punghorst, Former Chemist, National Training Center, EPA, WPO, Cincinnati, OH 45268, and revised by Charles R. Feldmann, Chemist, National Training Ctr.

Descriptors: Chemical Analysis, Chlorinated Hydrocarbon Pesticides, Gas Chromatography, Laboratory Tests, Pesticide Residues, Pesticides, Two Four D, Water Analysis

TOTAL CARBON ANALYSIS

I INTRODUCTION

A History of Carbon Analyses

In the wake of a rapid population growth, and the increasing heavy use of our natural waterways, the nation, and indeed the world, is presented with the acute problem of increased pollutional loads on streams, rivers and other receiving bodies. This has resulted in a growing awareness of the need to prevent the pollution of streams, rivers, lakes and even the oceans. Along with this awareness has developed a desire for a more rapid and precise method of detecting and measuring pollution due to organic materials.

B The Methods

In the past, two general approaches have been used in evaluating the degree of organic water pollution.

1 The determination of the amount of oxygen or other oxidants required to react with organic impurities.

2 The determination of the amount of total carbon present in these impurities.

C Oxygen Demand Analyses

The first approach is represented by conventional laboratory tests for determining Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD). One of the principal disadvantages of these tests is that they are limited primarily to historical significance, that is, they tell what a treatment plant had been doing, since they require anywhere from two hours to five days to complete. Since up to now no faster method has been available, traditional BOD and COD determinations have become accepted standards of measure in water pollution control work even though they are essentially ineffective for process control purposes.

Until the introduction of the Carbonaceous Analyzer, all methods taking the second approach, the total carbon method of evaluating water quality, also proved too slow.

II THE ANALYSIS OF CARBON

A Pollution Indicator

Now the carbonaceous analyzer provides a means to determine the total carbon content of a dilute water sample in approximately two minutes. With proper sample preparation to remove inorganic carbonates, the instrument determines the total organic carbon content in the sample.

B Relationship of Carbon Analysis to BOD and COD

This quantity varies with the structure from 27 percent for oxalic acid through 40 percent for glucose to 75 percent for methane. The ratio of COD to mg carbon also varies widely from 0.67 for oxalic acid through 2.67 for glucose to 5.33 for methane. Representative secondary sewage effluents have given a ratio of COD to carbon content of between 2.5 and 3.5 with the general average being 3.0.

The BOD, COD and carbon contents of these and some other representative compounds are summarized in the following table.

Sample	5-Day BOD-mg/mg	COD-mg/mg	% Carbon
Stearic Acid - $C_{18}H_{36}O_2$	.786	2.91	76
Glucose - $C_6H_{12}O_6$	.73	1.07	40
Oxalic Acid - $C_2H_2O_4$	.14	.18	27
Benzoic Acid - $C_7H_6O_2$	1.38	1.97	69
Phenol - C_6H_6O	.05 to 2.1 depending upon concentration	2.36	77
Potassium Acid Phthalate $KHC_8H_4O_4$	.95	1.15	47
Salicylic Acid - $C_7H_6O_3$	1.25	1.60	61
Secondary Effluent, Clarified	13*	75*	21*
" " "	23*	67*	12*
" " "	4*	36*	7*

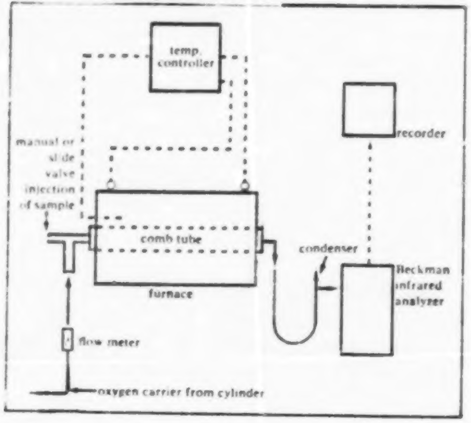
*In units of mg/l

III THE CARBON ANALYZER

A Principle of Operation

Basically the carbonaceous analyzer consists of three sections - a sampling and oxidizing system, a Beckman Model 315 Infrared Analyzer, and a strip-chart recorder.

Carbonaceous Analyzer Schematic


temp. controller
recorder
manual or slide valve injection of sample
comb tube
condenser
furnace
Beckman infrared analyzer
flow meter
oxygen carrier from cylinder

A micro sample (20-40 µl) of the water to be analyzed is injected into a catalytic combustion tube which is enclosed by an electric furnace thermostated at 950°C. The water is vaporized and the carbonaceous material is oxidized to carbon dioxide (CO_2) and steam in a carrier stream of pure oxygen. The oxygen flow carries the steam and CO_2 out of the furnace where the steam is condensed and the condensate removed. The CO_2, oxygen and remaining water vapor enter an infrared analyzer sensitized to provide a measure of CO_2. The output of the infrared analyzer is recorded on a strip chart, after which, the curve produced can be evaluated by comparing peak height with a calibration curve based upon standard solutions. Results are obtained directly in milligrams of carbon per liter.

B Application

Results(1) show that the method is applicable for most, if not all, water-soluble organic compounds -- including those that contain sulfur, nitrogen, and volatiles.

Nonvolatile organic substances can be differentiated from volatiles, such as carbon dioxide or light hydrocarbons by determination of carbon both before and after the sample solution has been blown with an inert gas.

C Sample Preparation

The Carbonaceous Analyzer is often referred to as a total carbon analyzer because it provides a measure of all the carbonaceous material in a sample, both organic and inorganic. However, if a measure of organic carbon alone is desired, the inorganic carbon content of the sample can be removed during sample preparation.

1 Removal of inorganic carbon

The simplest procedure for removing inorganic carbon from the sample is one of acidifying and blowing. A few drops of HCl per 100 ml of sample will normally reduce pH to 2 or less, releasing all the inorganic carbon as CO_2. Five minutes of blowing with a gas free of CO_2 sweeps out the CO_2 formed by the inorganic carbon. Only the organic carbon remains in the sample and may be analyzed without the inorganic interference.

2 Volatile carbonaceous material

Volatile carbonaceous material that may be lost by blowing is accounted for by using a dual channel carbon analyzer. Beckman's new analyzer has the previously detailed high temperature (950°C) furnace plus a low temperature (150°C) one. Using quartz chips wetted with phosphoric acid, the low temperature channel senses only the CO_2 (freed by the acid) in the original sample. The remaining organics and water are retained in the condenser connected to this low temperature furnace. None of the organics are oxidized by the 150°C furnace.

By injecting a sample into the low temperature furnace, a peak representing the inorganic carbon is obtained on the strip chart. Injecting a nonacidified sample into the high temperature furnace yields a peak representing the total carbon. The difference between the values determined for the two peaks is the total organic carbon.

3 Dilute samples

If the sample is dilute (less than 100 mg/liter carbon) and is a true solution (no suspended particles) no further preparation is required.

4 Samples containing solids

If the sample contains solids and/or fibers which are to be included in the determination, these must be reduced in size so that they will be able to pass through the needle which has an opening of 170 microns (needles having larger openings may be obtained if necessary). In most cases, mixing the/sample in a Waring Blender will reduce the particle size sufficiently for sampling.

IV PROCEDURE FOR ANALYSIS

A Interferences

Water vapor resulting from vaporization of the sample, causes a slight interference in the method. Most of the water is trapped out by the air condenser positioned immediately after the combustion furnace. However, a portion of the water vapor passes through the system into the infrared detector and appears on the strip chart as carbon. The water blank also appears on the standard calibration curve, and is therefore removed from the final calculation. In tests of solutions containing the following anions: NO_3^-, Cl^-, SO^{-2}, PO_4^{-3}, no interference was encountered with concentrations up to one percent.

B Precision and Accuracy

The recovery of carbon from standard solutions is 98.5 - 100.0 percent. The minimum detectable concentration using the prescribed operating instructions is 1 mg/l carbon. Generally, the data are reproducible to ± 1 mg/l with a standard deviation of 0.7 mg/l at the 100 mg/l level

V APPLICATIONS

Several of the many research and industrial applications of the Carbonaceous Analyzer are listed below:

A Determine the efficiency of various wastewater renovation processes, both in the laboratory and in the field.

B Compare a plant's waste outlet with its water inlet to determine the degree of contamination contributed.

C Monitoring a waste stream to check for product loss.

D Follow the rate of utilization of organic nutrients by micro-organisms.

E To detect organic impurities in inorganic compounds.

VI ADVANTAGES OF CARBON ANALYZER

A Speed

The Carbonaceous Analyzer's most important advantage is it's speed of analysis. One analysis can be performed in 2-3 minutes. This speed of analysis brings about another advantage, economy of operation. Working with dilute samples, one man can run ten to twenty carbon determinations per hour. This is probably more than the number of COD or BOD tests that can even be started, much less completed, in the same period of time.

B Total Carbon

Another advantage is that the measure of carbon is a total one. The oxidizing system of the analyzer brings about complete oxidation of any form of carbon. No compound has been found to which the method is inapplicable.

VII CONCLUSIONS

The Carbonaceous Analyzer provides a rapid and precise measurement of organic carbon in both liquid and air samples. It should be found useful for many research and industrial applications, a few of which have been mentioned.

Because of its rapidity it may be found more useful than the more time-consuming BOD and COD measurements for monitoring industrial waste streams or waste treatment processes.

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This outline was prepared by Robert T. Williams, Chief, Analytical Applications Laboratory, AWTL, NERC, Cincinnati, OH 45268 and revised by Charles J. Moench, Jr., Waste Identification and Analysis, AWTL, NERC, Cincinnati, OH 45268.

Descriptors: Biochemical Oxygen Demand, Carbon, Chemical Analysis, Chemical Oxygen Demand, Organic Matter, Organic Wastes, Water Analysis

CARBON-NITROGEN-HYDROGEN ANALYZER

I INTRODUCTION

Over the years, the classical Pregl and Dumas procedures[3, 5] for the microdetermination of carbon, hydrogen, and nitrogen provided the microchemist with reliable analytical data. However, the disadvantages of these traditional methods:

- TIME-CONSUMING PROCEDURE - about 45 minutes is required for a complete carbon, hydrogen, nitrogen analysis.
- SPECIAL LABORATORY FACILITIES - for reliable microanalytical results, a filtered air atmosphere, in a vibration-free, temperature and humidity controlled environment is required.
- SPECIAL PRECISION EQUIPMENT - measurement of small, but real, differences in the weight of gas absorption tubes requires a high sensitivity, high capacity microbalance and a skilled operator.
- HIGHLY SKILLED TECHNICIANS - because of the delicate and tedious operations involved, unusually competent technicians are essential.
- HIGH COST/ANALYSIS - due to above factors,

were also well known to the microchemist. Recognizing that the high cost/analysis was due to the expense involved in creating the necessary physical environment, and training competent technicians as well as the time lost by the researcher waiting for results, various instrumental methods for automating the Pregl and Dumas procedures were developed.

The acceptability of these innovations to the microchemist was determined, for the most part, by the recognized analytical limitations, or allowable errors, of the analyses.

As Dr. A. Steyermark[6] pointed out, "...the determination of carbon and hydrogen is always in order but is of no value if one is trying to differentiate between two compounds having these values so close together that they fall within the limits of allowable error. For example, suppose two such compounds have theoretical values of the following:

(a)	Compound I	Compound II
	C = 66.23%	C = 65.73%
	H = 4.76%	H = 4.36%

If a carbon-hydrogen determination gave

C = 66.03%   H = 4.56%

it would be impossible from this to say which of the two compounds, I or II, had been analyzed since the allowable error on both carbon and hydrogen are ± 0.3%." Obviously, with an allowable error of ± 0.3%, experimentally determined carbon values cannot be used to identify adjacent members of a homologous series of organic compounds.

In practice, the allowable error of ± 0.3% is accepted as the criterion for evaluating the reliability of duplicate test results and the analytical methods by which such values are obtained. However, the accepted 0.3% difference between duplicates, which varies somewhat from laboratory-to-laboratory or method-to-method, may not be the best criterion for evaluating reliability, or for comparing data from different sources. This concept is discussed more fully in the Data Evaluation Section.

In the late 1950's two instruments designed to overcome some of the problems involved in the microdetermination of carbon, hydrogen and nitrogen were placed on the market. To determine all three elements two instruments were required; one for carbon and hydrogen, and the other for nitrogen. While both instruments were designed for the automation of the basic Pregl and Dumas methods, only the sample combustion step was automated. But, the tedious and delicate weighing of the sample and the relatively heavy carbon dioxide and water absorption tubes, before and after combustion, was still a manual operation requiring a skilled technician. As with the carbon and hydrogen analyzer, the combustion step in the Dumas method was automated, but the sample weighing operation was still a manual one. However, a specialized nitrometer for measuring the gas volume directly in cubic centimeters was included in the nitrogen analyzer. With this procedure, nitrogen analyses could be performed in 10 minutes.

However, with today's rapid technological advances, a simpler and more fully automated method for the microdetermination of carbon, hydrogen and nitrogen was necessary to meet the demand for more efficient laboratory operations. Several investigators[2] described instrumental methods to measure the products of combustion, and gas chromatographic techniques[1, 4, 7, 8] were also used extensively with good results. Later, commercial gas chromatographic instruments were placed on the market. In one of these, combustion takes place in a flowing stream of oxygen rather than in the recommended statis system and water must be converted to acetylene prior to injection into the single column of the chromatograph. The components are frozen-out and then injected in the chromatograph.

In a second instrument, a mixture of helium and oxygen is used as the carrier gas. After combustion is complete, oxygen is removed from the carrier gas by reaction with metallic copper while the combustion products (CO_2, H_2O and N_2) are selectively separated in the detection section of the instrument. The presence of gases other than carbon dioxide, nitrogen, and water in the effluent stream will be measured as either CO_2 or N_2, and result in high values for these constituents.

The F & M Model 180 C-H-N Analyzer, which involves a modified Dumas technique for combustion, helium as the carrier gas, and a dual-column, dual-detection system, was designed to provide optimum sample-oxidant contact for combustion under static conditions, thus eliminating certain operational problems inherent in the other commercially available C, H and N instruments. The simplicity of operation, plus elimination of multiple weighings on a high-capacity microbalance enables a laboratory technician to obtain reliable results in a minimum of time.

II BASIC COMBUSTION CHEMISTRY

Regardless of the method used to determine carbon, hydrogen and nitrogen in an organic material, the chemistry involved is basically the same. The sample is decomposed by heat, and the products are oxidized -- carbon is converted to carbon dioxide, hydrogen to water and combined nitrogen to the free gas -- as shown in the following general reactions:

$$\text{Organic Sample} \xrightarrow[\text{Inert Atm.}]{\Delta + \text{cat.}} \text{Decomposition Products}$$

$$\text{Decomposition Products} \xrightarrow[\text{Inert Atm.}]{\Delta + \text{cat.}} CO_2 + H_2O + N_2$$

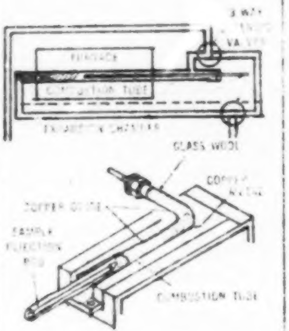
The optimum combustion conditions, established by Professor W. Simon[9] require adequate temperatures, an oxygen atmosphere, a suitable oxidation catalyst and a static system. These are recognized as critical factors in the design of instrumental systems for carbon, hydrogen, and nitrogen analyses. In recently developed commercially available instruments, oxygen, or an oxygen (3%) - helium mixture, is used as the carrier gas. The latter systems also provide for static conditions during combustion, whereas, with oxygen alone, a continuous gas flow is maintained. Since large volumes of carrier gas are required when the oxygen is limited to that in the oxygen (3%) - helium mixture, a system in which sufficient oxygen is produced under static conditions during combustion would simplify the mechanics and improve the efficiency of this procedural step. Such a system is an integral part of the recently developed Model 180 Analyzer.

In the Model 180 instrument, the sample elements are converted to the desired oxidation state (CO_2, H_2O and N_2) by means of a Ag_2O-MnO_2 catalyst-oxidant under static conditions. The oxidation reaction takes place in the combustion zone of the furnace section; see Figure 1.

Figure 1

SAMPLE INJECTION & COMBUSTION SYSTEMS

F&M MODEL 180 ANALYZER



The general reactions in the furnace (700-1000° C) are as follows:

$$Ag_2O + MnO_2 \xrightarrow{\Delta} 2\,Ag + MnO + O_2$$

$$\text{Sample} + O_2 \xrightarrow{\Delta} CO_2 + H_2O + N_2$$

An oxygen atmosphere in the combustion area is produced by decomposition of the silver oxide in the oxidant:

$$2\,Ag_2O \xrightarrow{300^\circ C} 4\,Ag + O_2$$

Then as the decomposition temperature of MnO_2 is reached, oxygen is also released in the immediate vicinity of any non-volatile pyrolytic compounds thus ensuring complete oxidation of the sample decomposition products.

$$2\,MnO_2 \xrightarrow{500^\circ C} 2\,MnO + O_2$$

The various oxides of carbon and/or nitrogen are converted to carbon dioxide and nitrogen by reaction with the CuO-Cu packing in the modified Dumas combustion tube.

$$CuO + CO \xrightarrow{\Delta} CO_2 + Cu$$

$$Cu + \text{Nitrogen Oxides} \xrightarrow{\Delta} CuO + N_2$$

Excess oxygen in the combustion tube is removed by the hot reduced copper before the effluent gases are admitted to the chromatographic section.

$$2\,Cu + O_2 \xrightarrow{\Delta} 2\,CuO$$

After carbon, hydrogen and nitrogen are converted to the desired oxidation state by combustion, the quantity of each can be determined by gas chromatography or by some other method. Regardless of the method selected for making the final measurements, the chemistry involved up to that point is essentially the same.

III DESIGN CONCEPT

Basically, the F & M Model 180 Carbon, Hydrogen and Nitrogen Analyzer was designed to overcome the obvious disadvantages of the classical Pregl and Dumas methods. This necessitated the development of systems to:

- Replace the multiple weighing manipulations which require a high-capacity, environment-sensitive microbalance and highly skilled technicians.

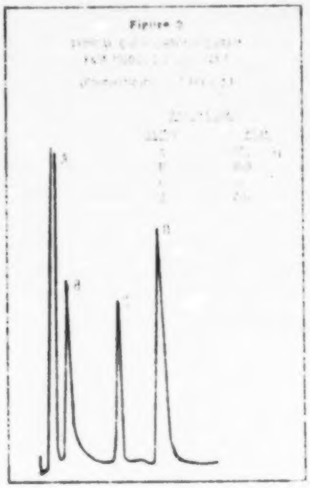
- Decrease the time required for a complete carbon, hydrogen and nitrogen sample analysis.
- Permit the simultaneous determination of carbon, hydrogen and nitrogen in a single sample.
- Simplify the computations.

The first of the design objectives was achieved by replacing the conventional microbalance with a low-capacity, environment-insensitive electro-microbalance and eliminating the heavy adsorption tubes. Also, all operations are performed under normal laboratory conditions thus eliminating the need for the special laboratory environment required for conventional microanalysis.

In order to decrease the time required for a conventional microanalysis and to permit the simultaneous determination of carbon, hydrogen and nitrogen in a single test portion, a modified Dumas combustion technique and a dual-column, dual-detector gas chromatograph were combined to give a semi-automatic system. The instrument consists of three sections:

- An injection system for introducing the sample into the hot zone of the furnace.
- A furnace section where the sample is oxidized and the reaction products are stored prior to injection into the chromatographic columns.
- A chromatograph section where the reaction products are separated into individual components and measured.

As the individual components pass through the dual-column, dual-detector portion of the chromatograph, they are separated into bands which appear as peaks on the recorder chart, see Figure 2. The concentration of each component is then calculated from peak-height measurements; under certain conditions, calculations may be based on peak-area measurements. The time required for a complete carbon, hydrogen and nitrogen sample analysis is about 10 minutes as compared to 45-80 minutes by conventional microanalytical methods.



In the design of the M-180, a Cahn Ratio Electrobalance^R is combined with a Ratio-Recording System to electrically compensate for the variation in recorder response due to changes in sample weight. Since this combination in effect divides the recorder signal by the sample weight, the peak height for a given compound then becomes a constant. The advantages of this Electrobalance-Recorder combination include:

1 Convenience in checking instrument performance.

2 Elimination of errors in reading and recording sample weights.

3 Simplification of computations.

The mechanism involved in electrically compensating for the recorder response to sample weight is as follows: (1) a voltage, proportional to sample weight, is applied across the slidewire of the recorder as the sample is weighed on the Electrobalance; (2) recorder sensitivity which varies inversely with sample weight automatically adjusts to compensate for the input voltage from the Electrobalance;

(3) the compensated recorder signal thus becomes a function of sample composition and is independent of sample weight.

The reproducibility of peak height measurements with the Ratio-Recording System in operation is shown in Figure 3 for four samples, while the precision of the carbon, hydrogen, nitrogen values, derived from the chromatograms, are presented in Table 1.

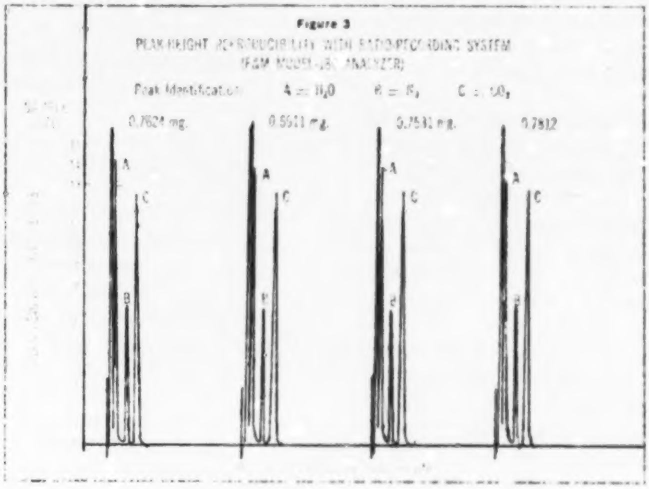


Figure 3

PEAK HEIGHT REPRODUCIBILITY WITH RATIO-RECORDING SYSTEM (F&M MODEL 180 ANALYZER)

TABLE 1

Reproducibility of Peak-Height Measurements
With Ratio-Recording System
(F & M M-180 C-H-N Analyzer)

Samples	Sample Weight (mg)	Peak-Height Measurements Carbon	 Hydrogen	 Nitrogen
		(Centimeters)		
1	0.7624	12.60	14.05	6.62
2	.6911	12.59	13.36	6.65
3	.7531	12.58	13.78	6.51
4	.7812	12.60	13.25	6.65
Average - $\bar{X}$		12.59	13.61	6.60
Range - R		0.02	0.80	0.14
Std. Deviation - S		.01	.12	.07
Conversion Factor K*		4.11	.372	3.06

*$K\bar{X}$ = C, H, N Values in Percent.

Calibration curves for both peak height and peak area are prepared by processing appropriate-size weighed portions of a standard sample (a material of known composition), and plotting the peak-heights or peak-areas recorded for the reaction products, CO_2, H_2O and N_2 against the corresponding known values for carbon, hydrogen and nitrogen. Typical peak-height and peak-area calibration curves are shown for carbon in Figure 4.

At higher carbon values, (see Figure 4), the peak-height calibration curve is no longer linear. Furthermore, the Ratio Recording System cannot be used with larger sample sizes; above 0.8 milligrams, the detection system is overloaded to such an extent that the relationship between peak-height and component concentration is no longer linear. In these cases, the Ratio Recording System is cut out of the recorder circuit and test results are calculated from specially prepared peak-height calibration curves or from peak-area measurements; under these circumstances, sample weights must be recorded. Obviously, the computational convenience associated with the use of the Ratio Recording System and peak-height measurements more than compensates for any inconvenience involved in adjusting the sample size to the optimum weight.

With the F & M Model 180 Analyzer a simultaneous determination of carbon, hydrogen and nitrogen in the sample weight range of 0.5 to 1 mg can be completed within 10 minutes; with the Ratio Recorder disconnected, samples as small as 0.25 mg can be analyzed. Only a single sample is required for the complete analysis and all analyses are made under normal laboratory conditions. Consequently, the obvious disadvantages of the classical Pregl and Dumas methods are eliminated.

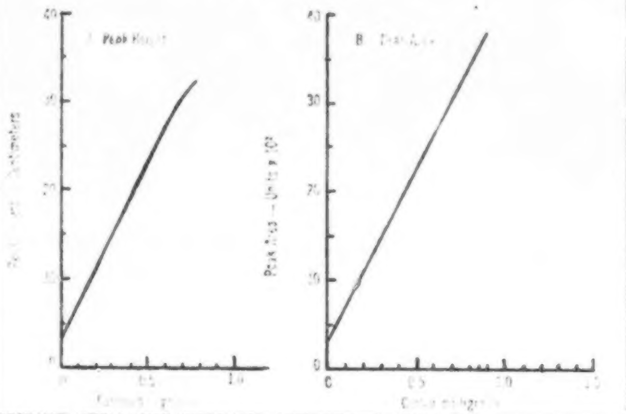
IV OPERATIONAL FEATURES

In the Model 180 Analyzer, the bypass carrier gas system and expansion chamber permit variation in the time required for complete oxidation of the sample and also provide partial cooling and storage of the reaction product. The combustion temperature, ambient to 1000° C, is controlled with an F & M Variable Power Regulator (VPR) and the combustion tube is packed with cupric oxide and reduced copper wire to ensure complete oxidation and reduction. With this combustion system, most samples are completely oxidized in 2 minutes at 800° C.

Figure 4

PEAK HEIGHT AND PEAK AREA CALIBRATION CURVES

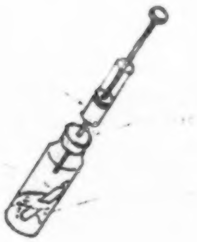
F&M MODEL 180 ANALYZER



The injection system, through which the sample is placed in the combustion area, was designed to minimize dead volume and to maintain a gas tight seal during sample processing. Because of the sample-injection and combustion-system design, operating temperatures can be maintained on a continuous basis. Thus, the need for a periodic cooling cycle is eliminated.

Solid samples are weighed on an Electrobalance in preformed, preconditioned (washed in organic solvent and heated at 200-500° C for 12 to 18 hours) aluminum boats which are made from one-mil aluminum foil. Prior to injection into the combustion system, the sample in the boat is covered with a layer of catalyzed oxidant to provide contact oxidation conditions in the combustion area.

Liquid samples, contained in thin-wall aluminum capillary tubes, are introduced into the M-180 C-H-N Analyzer in the same manner as solids. Prior to injection into the Analyzer, an empty pre-weighed capillary tube is placed in a small vial of the liquid sample (see Figure 5) and filled by creating a partial vacuum in the tightly sealed container. The loaded capillary is then sealed and reweighed to obtain the sample weight. While the liquid sample loading and weighing procedure is not as simple as that for handling solids, reliable carbon, hydrogen and nitrogen values are obtainable using this sampling technique.



Since an inert carrier gas, such as helium, is necessary to achieve the desired detection sensitivity, a separate oxidant with the following characteristics is required:

- Must be sufficiently powerful to oxidize the sample completely.
- Oxidation must be rapid and quantitative.
- The catalyst must be pure and free from CO_2, N_2 or H_2O contamination.

For these reasons, Ag_2O--MnO_2 was selected as the oxidant in the C-H-N procedure.

After combustion is complete, water in the reaction gases is separated from carbon dioxide and nitrogen in Column I, which is packed with Carbowax 1500 on Haloport F* support, and then measured (sensed) by Thermal Conductivity Detector I. The water is then removed from the gas stream by a Dow Dessicant 812 ^{R} drier at the head of a hold-up column which is provided to delay the elution of carbon dioxide and nitrogen until the water peak is fully developed.

Nitrogen is separated from carbon dioxide in Colunm II, which is packed with granulated Pittsburgh Coke and Chemical BPL activated carbon*. Then, each gas is measured as it passes through Thermal Conductivity Detector II. The signals developed by the Thermal Conductivity detectors, as the gases pass through the columns, are recorded on a Brown Instrument Recorder.

The entire chromatographic system is operated isothermally by housing the components in an insulated oven which is maintained at a constant temperature of 100-150° C. Cartridge heaters and a thermoswitch embedded in a one-inch aluminum plate, which extends over the entire oven base, maintain the oven temperature at the desired level.

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*Available from F & M Scientific Corporation, Avondale, Pennsylvania.

V DATA EVALUATION

In microanalytical work, an allowable error of ± 0.3%[6] is the accepted reliability criterion for carbon, hydrogen and nitrogen analyses. As applied by the microchemist, duplicate test results for any of the three constituents are considered acceptable if the difference (D) does not exceed this allowable error. Consequently, the accepted or allowable error is a recognized measure of the precision or reproducibility of values obtained by a given procedure and can be used as a comparison criterion for evaluating different methods of analysis. In other words, the allowable error of ± 0.3% defines the limits within which differences in duplicates are expected to lie and thus is considered as the accepted limit of analytical error for carbon, hydrogen and nitrogen analyses.

However, the limit of error for these analyses can also be derived from the standard deviation (S) which is a generally recognized term for expressing precision or reliability. The formula for limit of error (L. E.) is as follows:

$$\text{L.E.} = \pm KS$$

where

S = Standard deviation

K = Probability level factor based on the area under the Normal Distribution curve. For practical purposes,

$K = 1 = 67\%$ probability level, or the area under the curve designated as $\bar{X} \pm S$.

$K = 2 = 95\%$ probability level, or the area under the curve designated as $\bar{X} \pm 2S$.

$K = 3 = 99\%$ probability level, or the area under the curve designated as $\bar{X} \pm 3S$.

$K = 4 = 100\%$ probability level, or the area under the curve designated as $\bar{X} \pm 4S$.

Now, the relationship[10, 11] between standard deviations (S), and the acceptable difference (D) or range (R) in duplicates, is as follows:

$$S = (R_f)$$

when $R = D$,

$$S = D\,(R_f)$$

where:

S = Standard deviation

R = Range or difference between high and low values in a set of data

D = Difference between duplicates

R_f = Conversion factor for duplicates = 0.885

So, if

D = Allowable Error...and the Allowable Error = ± 0.3%

then,

$$D = 0.3\%$$

and

$$S = 0.30 \times 0.885$$
$$= 0.27\%$$

Thus, the limit of error, depending on the probability level selected, becomes

$$\begin{aligned} \text{L.E.} &= \pm 0.27\% \\ &= \pm 0.54\% \\ &= \pm 0.81\% \\ &= \pm 1.08\% \end{aligned}$$

Obviously, the limit of error (based on the standard deviation) at a 67% probability level is equivalent to the accepted limit of analytical error for carbon, hydrogen, and nitrogen analyses. Thus, only two-thirds of the difference between duplicates can be expected to lie within the accepted limits of analytical error. Therefore, an evaluation criterion based on the limit at a 95% probability level is recommended.

The data summarized in Table 2 for laboratories 1 thru 6 were derived from duplicate Pregl values published in the Journal of the American Chemical Society for a one-year period. While the average difference between duplicates varies somewhat from laboratory to laboratory, the overall difference between duplicates (0.24) is within the limit of error at a 95% probability level for both carbon and hydrogen. Furthermore, evaluation of F & M Model 180 data, see Table 2, yielded a comparable overall difference between duplicates. Therefore, test results of comparable precision, or reliability, are obtainable by the Pregl and the F & M Model 180 Analyzer.

TABLE 2

Reproducibility[a] of F & M M-180 & Pregl Data

		Carbon Data			Hydrogen Data		
Method	Laboratory	Number of Duplicates (N)	Average Difference (D)	Standard Deviation (S)[c]	Number of Duplicates (N)	Average Difference (D)	Standard Deviation (S)[c]
M-180	A	44	0.27	0.24	44	0.10	0.09
	B	20	.19	.17	20	.13	.11
	Over-All	64	0.23	0.20	64	0.12	0.11
Pregl[b]	1	3	0.22	0.19	3	0.11	0.10
	2	10	.15	.13	10	.09	.08
	3	9	.33	.29	9	.15	.13
	4	6	.35	.31	6	.15	.13
	5	11	.27	.24	11	.11	.10
	6	4	.10	.08	4	.10	.09
	Over-All	43	0.24	0.21	43	0.12	0.11

a Based on duplicate determinations
b Published data
c $S = DR_f$ where $R_f = 0.885$ (Based on N = 2 for duplicate determinations)

VI APPLICATION DEVELOPMENT

Since the development of the Model 180 C-H-N Analyzer, this method has been used in both the F & M and customer laboratories to analyze a variety of organic materials. Operational-type carbon, hydrogen and nitrogen calibration curves, such as those used in these analyses, are shown in Figure 6 while an operational set of peak-height calibration data is given in Table 3. The excellent reproducibility of the system blank and calibration factors is indicative of the reliability of the carbon, hydrogen and nitrogen test results.

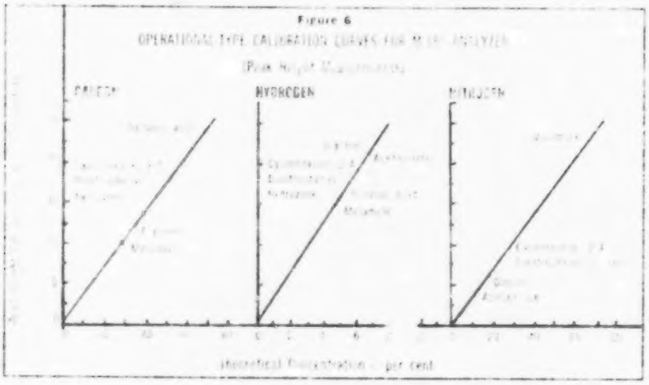
TABLE 3

C-H-N Peak Height Calibration Data
(F & M M-180 C-H-N Analyzer)

Standard: Cyclohexanone 2, 4 Dinitrophenylhydrazine
Composition: C = 51.79% H = 5.07% N = 20.16%

	Calibration Data*					
	Carbon		Hydrogen		Nitrogen	
Run No.	Peak Height (cm)	Factor (% C/cm) K_c	Peak Height (cm)	Factor (% H/cm) K_H	Peak Height (cm)	Factor (% N/cm) K_N
1	11.98	4.323	6.02	0.843	3.03	6.53
2	11.94	4.337	6.14	.827	3.08	6.53
3	11.98	4.323	6.20	.818	3.35	6.01
4	11.99	4.320	6.01	.843	3.08	6.54
5	11.98	4.323	6.00	.844	3.10	6.51
6	11.99	4.320	6.02	.843	3.09	6.52
7	11.94	4.337	5.96	.847	3.00	6.72
8	11.96	4.330	6.01	.845	3.02	6.67
9	11.94	4.337	5.90	.859	3.02	6.67
10	11.96	4.330	5.96	.850	3.05	6.62
11	11.96	4.330	6.09	.832	3.07	6.32
No. Measurements (N)		11		11		11
Average $\bar{X}$		4.328		0.841		6.51
Std. Deviation (S)		0.067		.011		0.41

*Blank = 2.00 for C, H_2 & N_2; Attenuation = 32.



After standard calibration curves are initially established with compounds of known composition, component factors (K) are derived in the following manner and are used for calculation purposes:

Carbon K_c =

$$\frac{\text{Percent Carbon in Standard}}{\text{Peak Height } CO_2 \text{ Peak--}CO_2 \text{ Blank}} = \% \text{ C/cm.}$$

Hydrogen K_H =

$$\frac{\text{Percent Hydrogen in Standard}}{\text{Peak Height } H_2O \text{ Peak--}H_2O \text{ Blank}} = \% \text{ H/cm.}$$

Nitrogen K_N =

$$\frac{\text{Percent Nitrogen in Standard}}{\text{Peak Height N--N Blank}} = \% \text{ N/cm.}$$

The reproducibility of the blank measurements and calibration factors from day-to-day is a convenient method for evaluating operator and instrument performance.

The type of results obtainable when peak-area measurements are used is shown in Table 4 while similar data based on peak-height measurements are presented in Table 5. From a comparison of the data in these two tables, it was concluded that:

- Computations based on peak-area and peak-height measurements yield equally valid test results.
- Sulfur and halogens in the sample apparently do not interfere with the analysis.
- No special sample handling is needed for a variety of sample types and different bondings.
- Low concentrations of carbon in combustible materials can be determined readily and without interference.

TABLE 4
C-H-N Peak-Area Data
(F & M M-180 C-H-N ANALYZER)

Sample Identification	Carbon			Hydrogen			Nitrogen		
	Known (Y)	Found* X (Percent)	S	Known (Y)	Found* X (Percent)	S	Known (Y)	Found* X (Percent)	S
Camphor	78.80	78.60	---	10.53	10.27	---	---	---	---
Polyacrylonitrile	68.00	67.90	0.15	5.70	5.70	0.16	26.90	26.50	0.10
5-Hydoxyindole Acetate[a]	63.30	63.40	.31	8.07	8.64	.08	8.42	8.36	.18
Homovanillic Acid	59.40	59.60	.34	5.49	5.39	.08	---	---	---
Glutamic Acid	40.80	40.80	.41	6.12	6.22	.27	9.56	9.37	.19
		40.80	.42		6.17	.23		---	---
Histidene-HCl·H_2O	34.44	34.90	.19	5.27	5.56	.26	20.10	19.60	.21
Arginine-HCl	34.20	34.10	.58	7.13	7.24	.17	26.55	26.10	.14
Glycine	31.99	32.60	.15	6.68	6.75	.06	18.67	18.30	.26
Cystine	29.69	30.50	.24	5.07	5.16	.15	11.78	11.60	---

NOTE: Data tabulated in descending order of carbon values.

* $\bar{X}$ = average; S= std. deviation; N = 10 or more measurements

[a] Cyclohexyl ammonium salt.

TABLE 5
C-H-N Peak-Height Data
(F & M M-180 C-H-N ANALYZER)

Sample Identification	Carbon			Hydrogen			Nitrogen		
	Known Y	Found* X (Percent)	S	Known Y	Found* X (Percent)	S	Known Y	Found* X (Percent)	S
Naphthalene	93.74	93.75	0.21	6.29	6.60	0.06	---	---	---
Cholesterol	83.87	83.81	.27	11.99	11.98	.23	---	---	---
Benzoic Acid	68.84	68.85	.18	4.95	4.86	.16	---	---	---
Triphenylphosphate	66.26	66.54	.22	4.63	4.69	.05	---	---	---
p-fluorobenzoic Acid	60.01	59.88	.19	3.60	3.80	.04	---	---	---
Phenylthiourea	55.19	55.26	.16	5.29	5.32	.14	18.41	18.20	0.2
S-Benzylthiouronium[a]	47.36	47.45	.22	5.47	5.51	.42	13.60	14.00	0.3
Hexachlorobenzene	25.30	25.20	.51	---	---	---	---	---	---
Sulfur	0.11	0.11	---	---	---	---	---	---	---
	.31	.27	---	---	---	---	---	---	---
	.85	.85	---	---	---	---	---	---	---

NOTE: Data tabulated in descending order of carbon values.

*Average ($\bar{X}$) and standard deviation (S) based on 10 or more measurements (i.e., N = 10).

[a] Chloride salt.

Because of the convenience and simplicity of the peak-height method for computing test values, a variety of organic materials were analyzed using the M-180 procedure and results were calculated on the basis of peak-height measurements; these data are tabulated in Table 6.

Since these samples present varying degrees of difficulty when analyzed for carbon and hydrogen by classical methods, the agreement between the theoretical content and that found by the M-180 procedure is considered very satisfactory. This work confirms the reliability of the F & M Model 180 C-H-N Analyzer.

By changing the carrier gas system to hydrogen and the combustion tube packing to a hydrogenation catalyst, the oxygen content of reducible metal oxides can probably be determined with the F & M Model 180 Analyzer.

TABLE 6
C-H-N Data for Variety of Organics
(F & M M-180 C-H-N ANALYZER)

Sample Identification[b]	Carbon		Hydrogen		Nitrogen	
	Theor.	Found[a]	Theor.	Found[a]	Theor.	Found[a]
	(Percent)		(Percent)		(Percent)	
Anthracene	94.34	94.36	5.66	5.99	---	---
Hexane[c]	84.60	84.75	16.38	16.80	---	---
Octane[c]	84.12	83.90	15.89	16.05	---	---
Triethylamine[c]	71.21	71.60	14.94	14.96	---	---
Unknown 2	69.13	69.05	11.35	11.54	3.51	3.15
Unknown 6	66.65	66.90	4.19	4.19	29.15	29.40
Triphenyl phosphate	66.26	66.57	4.63	4.69	---	---
Unknown 8	63.95	63.90	5.60	5.92	2.98	2.35
Unknown 9	62.90	62.80	2.84	2.72	11.30	10.65
Acetone[c]	62.04	62.45	10.41	11.05	---	---
p-fluorobenzoic acid	60.01	59.88	3.60	3.79	---	---
Acetonitrile[c]	58.51	58.70	7.37	7.34	---	---
Unknown 7	57.80	57.55	7.52	7.62	7.46	7.43
Unknown 10	59.69	59.39	5.73	5.64	4.97	4.55
Unknown 1	52.17	52.30	5.73	5.94	4.68	4.50
Potassium acid phthalate	47.05	47.10	2.47	2.69	---	---
Sulfanilamide	41.85	42.04	4.68	4.38	---	---
Unknown 4	37.93	37.75	2.65	2.80	22.10	21.80
2, 4-dinitrophenylhydrazine	36.37	36.54	3.05	2.89	---	---
Unknown 3	34.48	34.72	6.94	7.02	40.21	39.75
O-iodobenzoic acid	33.90	33.79	2.03	1.96	---	---
Unknown 5	33.39	33.30	1.68	1.59	15.56	15.35
Chloroform[c]	10.10	10.40	0.84	0.93	---	---

NOTE: Data tabulated in descending order based on carbon content.

[a] Average value based on 3 or more measurements.

[b] Unknown samples 1 thru 10 were unidentified organic materials submitted for analyses-theoretical values supplied at a later date.

[c] Liquid samples-average based on duplicate measurements.

In this method, the oxygen content of the sample would be calculated from the water peak-height measurement. However, in order to determine the oxygen content of organic materials, modification of the M-180 would be necessary.

VII SUMMARY

The classical Pregl and Dumas methods for the microdetermination of carbon, hydrogen and nitrogen in organic materials involve tedious, time-consuming micro-analytical techniques which require expensive, special laboratory facilities and highly skilled technicians. But, reliable test results are obtainable with these traditional procedures. With the Model 180 Carbon, Hydrogen, and Nitrogen Analyzer, however, equally acceptable analyses can be made simultaneously in about 10 minutes under normal laboratory conditions.

With this modern instrumental method, samples ranging in size from 0.5-1.0 mg can be analyzed with the Ratio-Recording System in operation. If the total carbon is greater than 0.60 mg, however, either the Ratio-Recording System must be cut out of the circuit and special peak-height calibration curves prepared, or peak-area measurements must be used to compute test values. Furthermore, samples of less than 0.25 mg can be analyzed but the Ratio-Recording System must be disconnected during the analysis. The applicability of the Model 180 to the analysis of such small samples is a decided advantage when only small quantities of an unusual or rare material are available and only a limited amount can be provided for analysis.

The sample injection and combustion section of the M-180 was designed to ensure complete combustion in a closed system. Furthermore, the reaction gases can be retained in the expansion chamber of the system, if necessary, while the combustion products from the preceding samples are being separated and measured in the chromatograph section of the instrument; this permits the operator to process samples on a continuous basis.

The chromatographic section of the M-180 instrument provides a rapid, automatic method for separating and eluting each constituent and for measuring the amount of the individual sample components. This permits the simultaneous determination of carbon, hydrogen and nitrogen, without additional weighings or sample handling. Since this automated process replaces many of the tedious, time-consuming operations of the older conventional micro-methods, the advantages inherent in the F & M Model 180 procedure are readily apparent.

Since the development of the F & M Model 180 Carbon, Hydrogen and Nitrogen Analyzer, problems such as the following have been handled successfully:

- Establishing empirical formula for organic compounds in chemical, petrochemical, biomedical, food, and agricultural laboratories.
- Concentration of carbon in spent catalyst.
- Boiler efficiency as a function of coal composition.
- Ratio of carbon to hydrogen in tar.
- Carbon content of refined sulfur.

Furthermore, the validity of the carbon, hydrogen, and nitrogen test results is not affected by sulfur or halogens in a sample.

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This outline was prepared by Orville N. Hinsvark, Philip B. Beltz and John J. McFadden, F & M Instrument Company, Avondale, Pennsylvania.

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